Molecular velocity auto-correlations in glycerol/water mixtures studied by NMR MGSE method✩

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Abstract

Molecular dynamics in binary mixtures of water and glycerol was studied by measuring the spectrum of water velocity auto-correlation in the frequency range from 0.05 – 10 kHz by using the NMR method of modulated gradient spin echo. The method shows that the diversity of diffusion signature in the short spin trajectories provides information about heterogeneity of molecular motion due to the motion in the micro-vortexes of hydrodynamic fluctuation, which is especially pronounced for the mixtures with the low glycerol content. As concentration of glycerol increases above 10vol%, a new feature of spectrum appears due to interaction of water molecules with the clusters formed around hydrophilic glycerol molecules. New spectrum exposes a rate thickening of molecular friction, according to Einstein-Smoluchowski-Kubo formula, which inhibits rapid molecular motions and creates the conditions for a slow process of spontaneously folding of disordered poly-peptides into biologically active protein molecules when immersed in such a mixture. Keywords: molecular dynamics, glycerol/water mixture, NMR, gradient spin-echo, self-diffusion, molecular velocity auto-correlation

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1. Introduction

Although the earliest models of liquids as totally disordered structures have been replaced by models of a long range-disordered and short range-ordered systems where associations of molecules into clusters can form due to intermolecular interactions. However, there is still lack of understanding of molecular dynamic in liquids that plays an important role in biological systems, which refers primarily to liquids with hydrogen bonding, such as water, alcohol, glycerol, and their mixtures. In this liquids a not completely understood molecular mechanisms spontaneously folds a disordered poly-peptide into a unique structure of biologically active protein molecule. Glycerol-water (G/W) mixtures are particularly active in maintaining the structure of biologically active macromolecules, promoting protein self-assembly and in preventing organelle disruption by freezing. Despite massive research efforts these questions remain among the key unresolved issue in soft condensed matter physics, physical chemistry, materials science and biophysics.

The G/W mixtures have already been a subject of extensive research involving thermodynamic measurements, broadband dielectric measurement, NMR, infrared (IR), and Raman spectroscopy, etc. These studies reveal changes in the aqueous structure beyond the first neighbor level, but general properties of the G/W mixtures and how they affect the molecular dynamics are not revealed. They also show that macrophages are formed, because the glycerol is a small molecule of trihydric alcohol with large affinity to form 3D hydrogen-bonded network pervading the bulk of mixtures. Hydrogen bond energy between glycerol molecules (0.25 eV) and between water (0.19 eV) are lower than the binding energy between water and glycerol molecules (0.29 eV), leading to the molecular clusterization confirmed by the dielectric measurements with almost tri-fold increase of the activation enthalpy of 0.9 mol% G/W mixture compared to the pure water at $H = 16.2 \text{ kJ/mol}$.

In liquids the thermal molecular motion is impeded by interactions with
its neighbors. Velocity auto-correlation function (VAF) is a quantity containing information about the underlying processes of molecular interaction and dynamics [34]. The VAF, which is associated with a number of physical properties, such as thermal and mass diffusion, sound propagation, transverse-wave excitation, and can have either a single-particle or a collective nature, has a profound meaning in the statistical physics of fluids [30, 11, 53], but it is extremely difficult to measure. Some information can be obtained by neutron scattering [55, 37, 4] and light scattering [43], but a short time scale of these methods cannot extract information of its long time properties. It leads to the conclusion that the computer simulation of molecular dynamics seems to be the most suitable tool for the study of translation dynamics in molecular systems [24, 13].

The current understanding of molecular dynamics in G/W mixtures is derived from experimental results in combination with computer simulations. They can reproduce some macroscopic physical properties, but they largely depend on the chosen models. Thus, conclusions derived from models regarding molecular structure and dynamics remain unfinished [73].

A lot of effort has been devoted to understand the molecular translation dynamics in water, glycerol and G/W mixtures by measuring the self-diffusion coefficient, \( D \). Well known are the studies of molecular dynamics in liquids by measuring \( D \) in water by tracer technique [47], the measurements in the G/W mixtures by using the interferometric micro-diffusion method [50] and by NMR methods [70, 16]. The results of tracer technique is commonly used to calibrate the diffusion measurements done by other techniques, especially those obtained by the NMR gradient spin echo method [29, 10]. This method uses the magnetic field gradient \( \nabla |\mathbf{B}| = \mathbf{G} \) (MFG) to detect the translation displacement of molecules via uneven precession of their atomic nuclear spins. A variant of this method, the pulsed gradient spin-echo (PGSE), provides the signal attenuation proportional to the molecular mean squared displacement (MSD) in the interval between two consecutive MFG pulses [72, 60]. However, the PGSE measurements in water at different pressures and temperatures [33, 78] show values of \( D \) scattered beyond the experimental uncertainty [47]. Differences
are commonly assigned to inaccurately calibrated MFG or to the convection flows in liquids. However, differences may also due to the failure to observe properly some of experimental parameters as shown in the following. In the measurement of G/W mixture by the stimulated PGSE technique [40], only $D$ of water is obtained, since its contribution to the NMR signal is well distinguished by glycerol. The study proves the validity of the Arrhenius behavior and the Stockes-Einstein relationship between $D$ and viscosity $\eta$ in the range of room temperatures, but with deviations at temperatures close to the glass transition. The diffusion in G/W mixtures were measured also with the PGSE-FT NMR method by measuring the NMR hydrogen signal of $CH_2$, and $OH$ to which both water and glycerol contribute [16]. Since the proton exchange between hydroxy of glycerol and water is much faster than the time intervals of spin-echo sequence, $D$ of water component was calculated by taking into account numbers of $OH$ groups belonging to water and glycerol molecules. The mutual diffusion coefficient of G/W mixtures was also measured by the Gouy interferometric technique, but these results are substantially higher than those obtained by the interferometric micro-diffusion method [50] and lower than those measured by the holographic interferometry [69]. No evident reason for these discrepancies has been found [16].

According to the Einstein definition [21], $D$ is a time derivative of the molecular mean squared displacement (MSD) in a long time limit. When measured in a finite time interval, as in the case of the PGSE methods, $D$ can exhibit time dependence, because the initial velocity of labeled molecule, $v(t)$, may not be forgotten fast enough. Thus, $D$ obtained by this method may be different from that obtained by tracer techniques. The measurements in the finite time interval can result in a time-dependent diffusion described by the Green-Kubo formula [34]

$$D_{zz}(\tau) = \int_0^\tau \langle v_z(t)v_z(0) \rangle \tau dt = \frac{2}{\pi} \int_0^\infty D_{zz}(\omega) \frac{\sin(\tau \omega)}{\omega} d\omega,$$

which is the self-diffusion coefficient , $D_{zz}(\tau)$ for the motion along $z$-direction in the diffusion interval $\tau$. It is related to the power spectrum i.e. the velocity
autocorrelation spectrum (VAS) \( D_{zz}(\omega)_{\tau} = \int_{0}^{\tau} \langle v_z(t)v_z(0) \rangle_{\tau} \cos(\omega t) dt \), where \( \langle \ldots \rangle_{\tau} \) indicates the ensemble average over the particle trajectories in the interval \( \tau \).

Theories \[36, 76, 26, 35\] and simulations \[2, 3, 75\] predict a long-time asymptotic of the VAF with the \( t^{-3/2} \)-long time tail in liquids. Recent studies by the NMR modulated gradient spin-echo (MGSE) method \[67\] have shown a deviation from this asymptotic properties in simple liquids such as water, ethanol and glycerol due to inter-molecular interactions. In these measurements the unusual heterogeneity of molecular motion is observed, when the measurement interval is very short, which one cannot describe by a simple diffusion coefficient. In order to enlighten these phenomena and the function of glycerol as a colligative solute we set out to study the molecular self-diffusion in G/W mixtures by the NMR MGSE method presented in the following.

2. NMR gradient spin-echo method

In liquids rapid molecular motion on the time scale of pico- or nanoseconds completely nullifies the spin dipole-dipole and the first order quadrupole interactions, while the spin interactions with electrons in molecular orbitals and the electron mediated spin-spin interactions cannot be ignored. They appear as the chemical shifts and \( J \)-couplings in the NMR spectrum. Fluctuation of these interactions can be characterized by correlation functions of relevant physical quantities and affect spin relaxation. In the gradient spin echo method, the applied MFG is commonly strong enough that the effect of fluctuation of molecular translation velocity prevails in echo attenuation over all other interactions. Whenever the molecular displacements within the interval of measurement are shorter than the spatial spin phase discord, \( q(t) = \gamma \int_{0}^{t} G(t') f_\pi(t') dt' \), created by MFG and the train of \( \pi \)-RF pulses by switching \( b(t') \) in \( f_\pi(t) = \int_{0}^{t} \cos(b(t')) dt' \) between \( \pm \pi \) \[64\], the decay of signal of the spin-echo peak at the time \( \tau \) can be approximated by the cumulant series in the Gaussian approximation.
\[ E(\tau) = \sum_i E^{\alpha_i} e^{-i\alpha_i(\tau) - \beta_i(\tau)}. \]  

Here the sum goes over the sub-ensembles of spins with identical dynamical properties. The phase shift

\[ \alpha_i(\tau) = \int_0^\tau q(t) \cdot (v_i(t)) dt, \]

\[ \beta_i(\tau) = \frac{1}{\pi} \int_0^\infty q(\omega, \tau) D_i(\omega, \tau) q^*(\omega, \tau) d\omega, \]

where \( q(\omega, \tau) \) is the spectrum of the spin phase discord \( q(t) \) and where the VAS is

\[ D_i(\omega, \tau) = \int_0^\infty (v_i(t) \otimes v_i(0)) \tau \cos(\omega t) dt. \]

According to Eq. 4, the measurement of liquid by the pulsed gradient spin echo (PGSE) sequence, which consists of two MFG pulses of width \( \delta \) and separated for \( \Delta \), gives the spin echo attenuation in liquids

\[ \beta_i(\Delta, \delta) = \frac{\gamma^2 G^2}{\pi} \int_0^\infty D(\omega) \left( \frac{4 \sin(\omega \delta/2) \sin(\omega \Delta/2)}{\omega^2} \right)^2 d\omega, \]

from which \( D(\omega) \) and thus \( D(t) \) can be extracted with considerable difficulty by changing \( \Delta \) in the range of VAF correlation time \( \tau_c \). At the present state of the art, the MFG coil induction limits the width and the shape of the MFG pulses to above 1 ms, which is close to or slightly above values of \( \tau_c \) in some liquids. Thus, neglecting the dependence of the echo decay on \( \delta \) and \( \Delta \) according to Eq. 6 in the PGSE measurement of water gives an apparent self-diffusion coefficients that differ from one another and also deviate from those obtained from the theory and the simulations of molecular dynamics and water binary mixtures.
The determination of the long time asymptotic properties of VAF in dense systems is still a challenge that can be tackled by a NMR method, which directly probes the velocity auto-correlation spectrum (VAS). Such method is the modulated gradient spin echo [8], where the sequence of RF-pulses and MFG modulates the spatial dispersion of the spin phase. The method is basically a Carr-Purcell-Meiboom-Gill sequence (CPMG) consisting of initial $\pi/2$-RF-pulse and the train of $N \pi$-RF pulses separated by time intervals $T_{\text{10, 45}}$, which was used initially to reduce the effect of diffusion in the measurement of $T_2$ relaxation. Detailed analysis shows that the sequence imprints information about VAS, when applied in the combination with MFG [61, 62]. In first applications of MGSE method, pulsed or oscillating MFG were used to measure water flow through porous material [7] and molecular restricted self-diffusion in porous media [65, 4, 71, 51]. It was also demonstrated how the MGSE sequence improves the resolution of the diffusion-weighted MR images of the brain and the MRI of the diffusion tensor of neurons [1]. As in the case of PGSE, the frequency range of MGSE with the pulsed MFG is limited to below 1 kHz due to the gradient coil self-inductance. With the development of the MGSE technique in constant MFG, the frequency induction limit was avoided. New technique enables higher frequency limit, which is now determined by the power of the RF transmitter and the magnitude of the MFG, while the lowest limit is inversely proportional the spin relaxation time. The use of the MGSE techniques with fixed MFG was first proposed in reference [8] with concern about the side effects of using RF pulses in the presence of background MFG. Analysis of adverse interferences of both fields [64, 61] shows that at suitable experimental conditions the MGSE signal decay can be described as

$$E(\tau, \omega_m) = \sum_i E_{oi} e^{-\tau \frac{T_{2i}}{2}} \frac{\gamma^2 G^2}{\pi^2 \omega_m^2} D_{zz}(\omega_m, \tau) \tau. \quad (7)$$

Here, $\tau = NT$ and $D_{zz}(\omega_m, \tau)$ denotes the component of VAS tensor of the $i$-th spin sub-ensemble in the direction of applied MFG at the modulation frequency $\omega_m = \pi/T$ averaged over the interval $\tau$, and where $T_{2i}$ is the spin relaxation time.
The advantage of the new MGSE technique was demonstrated by measuring the VAS of restricted diffusion in pores smaller than 0.1 µm, by measuring the VAS of granular dynamics in fluidized granular systems, and by the discovery of a new low frequency mode of motion in polymer melts. Instead of using the externally applied MFG, this MGSE method also allows to employ MFG generated by the susceptibility differences on interfaces in porous systems to obtain information about the pore morphology and distribution of internal MFG.

3. Experiments

NMR spectrometer with 100 MHz proton Larmor frequency equipped with the Maxwell gradient coils to generate MFG in steps to the maximum of 5.7 T/m was used to measure the VAS of in G/W mixtures at room temperatures by the MGSE method. Only water echoes are traced, as the contribution of glycerol to signal decay can be neglected due to its long diffusion rate and short spin relaxation time. Results were checked by repeated measurements of the same samples on the NMR-MOUSE working at 18.7 MHz proton Larmor frequency with fixed MFG of 21.6 T/m. The high magnetic field of the 100 MHz spectrometer allows measurements with a high signal to noise ratio, but with the top frequency limited to 3 kHz due to the weak MFG. On the other hand, the large MFG of one-sided magnet of the NMR MOUSE allows measurement of very slow diffusion in the frequency range to about 10 kHz. However, its fix MFG limits the measurements on G/W mixtures below 1 kHz due to fast diffusion rate of water.

The samples of pure glycerol (99.5% Sigma-Aldrich), distilled water and mixtures with several different volume fractions of glycerol were prepared in plastic bottles of 100 ml volume. Tightly sealed with a plastic lid were kept for several days before used for the measurements. The samples were loaded into 15 mm long and 5 mm wide pyrex glass ampules and closed with paraffin tape to be inserted in the head of the 100 MHz NMR spectrometer. While the
Figure 1: MGSE spin echo decays in a mixture of 20 vol% glycerol in water mixture at different modulation frequencies ($\omega = \pi/T$). The inset picture shows the signal decay in zero MFG.

flat shelf of one-sided magnet of the NMR-MOUSE allows the measurements of mixtures in bottles without loading step. In order to avoid a possible impact of restricted diffusion containers with diameter much longer than the molecular displacements in the time of 100 ms long intervals of measurement were used. In addition, the effect of restricted diffusion is even further reduced by the initial $\pi/2$-RF pulse of the MGSE sequence applied in the background of MFG, because it excites only a few mm narrow slice of sample.

In the measurements on the 100 MHz NMR device, the amplitude of echo peaks is recorded in the time interval $\tau = 100$ ms by changing $T$ and magnitude of MFG, $G$, in a way to keep the product $T \times G = const$. Fig[1] shows that the number of recorded peaks increases with the shortening of $T$, while exponential decay of peaks with $\tau$ is effected only by the changes of $D(\omega, \tau)$, where $\omega = \pi/T$. The contribution of spin relaxation to the signal decay was determined by separate measurements in zero MFG as shown in the inset picture in the Fig[1]. The spin relaxation exhibits almost a clear mono-exponential decay, except in
Figure 2: 3D temporal-frequency plots of apparent VAS for the G/W mixtures with 0 vol%, 5 vol%, 10 vol%, 15 vol% of glycerol.
the initial interval of a few milliseconds, with the rapid decay believed to belong to water bounded in clusters.

4. Results and discussion

The MGSE measurements of G/W mixtures on the 100 MHz NMR device give time dependencies of spin-echoes that deviate from the anticipated mono-exponential decays particularly in pure water and G/W mixtures with lower glycerol contents. The deviations are clearly visible in the 3D frequency/temporal plots of the time derivatives of curves obtained by fitting the echo peaks with the fifth-order polynomial and with the coefficient of determination of $R^2 > 0.99999$ shown in Fig.2 and Fig.6. Instead of flat surface along the echo time, $\tau$, anticipated for the exponential decays, the deviations occur as a humped surface in the ranges of short $\tau$ and of higher frequencies. In the case of simple liquids [67], we interpreted similar humped surface by the molecular diffusion diversity due
to the motion in the vortexes of hydrodynamic fluctuation. Namely, at initial interval after the spin excitation, when the trajectories of the molecules are still short, the spins may observe local inhomogeneity caused by the distribution of internal MFG in the porous medium \[67\] or motional diversity due to fluxes or hydrodynamic oscillation in liquids \[64\], for example. By grouping the spin bearing particles into separate sub-ensembles corresponding to spins with the different spin-echo attenuation, we can describe the induction signal with the distribution function \(P(D)\) as

\[
E(\tau) = \int P(D)e^{-sD}dD
\]

with

\[
s = \frac{8\gamma^2 G^2}{\pi \omega^2}
\]

given in Eq.7. In the case of narrow distribution, the signal attenuation can be approximated by

\[
\log E = \beta(\tau) \approx -\tau/T_2 - s\langle D \rangle \tau + \frac{s^2}{2} \langle \Delta D^2 \rangle \tau^2 + ...
\]

(8)

Here \(\langle D \rangle\) is the mean diffusion coefficient and \(\langle \Delta D^2 \rangle\) is the variance of distribution. In the cases of a non-exponential decay the time derivatives of echo attenuation do not give VAS, but some apparent one, \(D_{app}(\omega, \tau)\), which conveys information the mean diffusion coefficient and its distribution. 3D frequency/temporal plots of \(D_{app}(\omega, \tau)\) for pure water and for G/W mixtures with 0.05, 0.10 and 0.15 volume fraction of glycerol are shown in Fig.2 and for the G/W mixtures with 0.20, 0.33, 0.50 and 0.66 volume fraction of glycerol in Fig.5.

In water and mixtures with the lower glycerol content a hump is evident in the 3D surface of spectra in the range of short \(\tau\) and at higher frequencies due to the distribution of the self-diffusion coefficients. The hump is clearly observable in the contour plot of the second derivative of \(\beta(\tau)\) of the mixture with 5 vol\% of glycerol content in Fig.3 which shows how \(\langle \Delta D^2 \rangle\) occurs at high modulation frequencies and disappears with increasing echo-time at \(\tau > 40\text{ms}\), when the trajectories of spins become long enough to span the whole extend of heterogeneity and to average off the diffusion diversity into \(\langle \Delta D^2 \rangle = 0\), what is also seen in Fig.2 and Fig.6. Similar diversity was observed in the MGSE measurements of VAS in water, ethanol and toluene with the explanation of the molecular self-diffusion in the vortexes of hydrodynamic fluctuation \[67\].

By increasing the glycerol content, the spectral hump is reduced, but in the
mixtures with $\geq 10$ vol% of glycerol a new spectral feature appears in the form of a low frequency ridge that levels at higher frequencies. In order to enhance visibility of spectral changes and to avoid the part affected by hydrodynamic fluctuation, where $\langle \Delta D^2 \rangle \neq 0$, we present in Fig.4 the VAS at echo times $\tau > 50$ ms for the mixtures with 0.00, 0.05, 0.10 and 0.15 vol% of glycerol. It shows the spectrum of mixture with 5 vol% of glycerol, which has a form similar to that of pure water, but only shifted upwards by about 30%. The upwards shift indicates a disruption of hydrogen bonding in water caused by a low glycerol content in water. A drastic change of spectra appears at the concentrations of 10 and 15 vol% of glycerol indicating new type of inter-molecular interaction, which has a strong impact on the molecular dynamics. 3D presentations of spectra in Fig.2 and Fig.5 clearly show how the increase of glycerol content lowers the spectrum level, decreases the spectral hump which is attributed to the bulk water. The low frequency ridge of the new spectrum, which starts to appear at 10vol% of glycerol, remains almost unchanged.

In order to ensure the validity of results obtained by the high frequency NMR device, the measurements of the samples were repeated by using the 18.7 MHz NMR MOUSE device. Its strong and fixed MFG enables the measurement of samples up to about 10 kHz, but with the lower S/N ratio due to lower Larmor frequency. It also does not allows the measurements of water and G/W mixtures below 1 kHz, due to excessive spin echo attenuation. Fig.6 shows the results of measurements on both devices, which match well in the overlapping frequency range.

4.1. Water-cluster interaction

In references [25, 28, 74, 56] a formation of clusters in the G/W mixtures was explained by the energies of bonding between water molecules and between glycerol molecules, which are lower than between water and glycerol molecules. Thus, the new spectral features of G/W mixture can be attributed to the formation of clusters around the hydrophilic glycerol molecules, which interaction with unbound water changes molecular dynamics. The spectral hump disap-
pearance with the increase of glycerol content indicates the depletion of the free water basin through the formation of new clusters. We believe that there are two contributions to the mixture VAS, when measuring proton signal by MGSE method: The contribution of free water that is not in contact with the clusters, and whose content decreases with the increase of glycerol concentration, and the water that interacts with the clusters or even could exchange with the clustered water. The VAS of pure glycerol and water bounded in the clusters cannot be detected due to a slow diffusion rate \(^{67}\) and the short relaxation time, and is only partially observed in Fig. 1 for the spin-echo decays in zero MFG.

At room temperatures the self-diffusion coefficient of G/W mixtures does not deviate significantly from Arrhenius’s law \(^{18}\), allowing to consider the molecular diffusion as the motion of particles occasionally caught up in potential wells created by their neighbors. Quite commonly the molecular dynamics is described by the Langevin equations, where the effect of the environment is taken into account through the fluctuating and frictional forces. The method has been widely used in the study of structural, thermal properties of matters in different phases \(^{58}\). However, the inter-molecular interaction and the coupling to other degrees of freedom are difficult to effectively include into it. The description of interaction between water-water, water-glycerol and glycerol-glycerol molecules with a simple Lennard-Jones potential turned out to be unsuccessful even though the potential is modified by the bifurcation of the single minimum into two or more minima \(^{59}\). However, the results of MGSE measurements in ordinary fluids \(^{77}\) showed that the results can be successfully explained by Langevin equations, in which we consider that molecular motion at high temperatures alters molecular interactions to such an extent that they can be approximated by harmonic interaction \(^{77}\). This means that in mutual collisions, the molecules occasionally get caught in a harmonic well, which can be described by a constant \(k\) and with a minimum at the \(a(t)\) between \(x_i\) and \(x_j\) locations of colliding molecules. This allows to describe the molecular dynamics
by the set of coupled Langevin equations (LE)

\[ m_i \frac{d^2 x_i}{dt^2} + \gamma \frac{dx_i}{dt} + k \sum_{j \neq i}^n (x_i - x_j - a(t)) = f_i(t) \] (9)

in which the \( i \)-th particle is coupled to \( n \) of its closest neighbors. Here, \( m_i \) is the particle mass, \( \gamma \) is the friction coefficient, and \( f_i \) is the random force. The friction and the random force represent two consequences of the same physical phenomenon and are interrelated

\[ \gamma(\omega) = \frac{1}{k_B T} \int_0^\infty \langle f(t)f(0) \rangle \exp (i\omega t) dt, \] (10)

where \( k_B \) is the Boltzmann constant. Neglecting the fluctuation of \( a(t) \) and the inertial terms of LE at low frequencies, when \( \omega < k/m_i \), and assuming that all particles are subjected to the same friction \( \gamma(\omega) \approx \gamma = \frac{1}{k_B T} \langle |f|^2 \rangle \), the solution gives the VAS of harmonically coupled water molecules, \( D_w(\omega) \), in the form

\[ D_w(\omega) = \frac{k_B T}{\gamma} \left( \frac{n + \tau_c^2 \omega^2}{n^2 + \tau_c^2 \omega^2} \right). \] (11)
where the correlation time is $\tau_c = \gamma/k$. At zero frequency, the Einstein diffusion coefficient, $D_w(0) = \frac{k_B T}{n\gamma}$, depends on the number of coupled molecules, while at high frequencies, $D_w(\infty) = \frac{k_B T}{\gamma}$ is the diffusion rate of molecules escaping the inter-molecular capturing. This formula provides a good fit to the VAS of pure water, glycerol and ethanol \[67\] as well also to the results of our measurements for the VAS of G/W mixture with 0.05 vol% glycerol shown in Fig.4. However, $D_w(\omega)$ cannot fit to the VAS of G/W mixture with the glycerol concentrations equal to or greater than 10 vol% as shown in the same picture. In the latter case, one must consider the interactions of water with the water clustered around glycerol. With a slight exaggeration that water molecules and clusters experience the same friction, $\gamma$, that the coupling constant between small and large particles is the same $k$, and given that the mass of clusters is large enough that the inertial term in their Langevin equations cannot be neglected at low frequencies, i.e. $\omega \approx \gamma/M$, the solution for for the system of $n_w$ light water molecules and $n_c$ heavy clusters gives the VAS for the water molecules interacting with clusters

$$D_{wc}(\omega) = \frac{k_B T}{\gamma n_w} \left( \frac{(n_w - 1)\tau^2\omega^2}{(n_c + n_w)^2 + \tau^2\omega^2} + \frac{n_c n_w + \tau^2\omega^2 + \left( n_w - \frac{\omega_o^2}{\omega_c^2} \right)^2}{(2n_c + n_w)n_c + \tau^2\omega^2 + \frac{n_c^2\omega^2}{\omega_c^2} + \left( n_w - \frac{\omega_o^2}{\omega_c^2} \right)^2} \right), \quad (12)$$

and the VAS for the clusters interacting with water

$$D_{cw}(\omega) = \frac{k_B T}{\gamma n_c} \left( \frac{(n_c - 1)\tau^2\omega^2}{(\omega_c^2/n_c - n_c - n_w)^2 + \tau^2\omega^2} + \frac{(n_c + n_w)n_c + \tau^2\omega^2 + \frac{n_c^2\omega^2}{\omega_c^2} + \left( n_c - \frac{\omega_o^2}{\omega_c^2} \right)^2}{(2n_c + n_w)n_c + \tau^2\omega^2 + \frac{n_c^2\omega^2}{\omega_c^2} + \left( n_c - \frac{\omega_o^2}{\omega_c^2} \right)^2} \right), \quad (13)$$

where $\omega_o = \sqrt{k/M}$. It might be an excessive simplification, but allows the calculation of which results would serve at least qualitatively to compare with our experimental results. To our surprise, Fig.4 shows a good fit of $D_{wc}(\omega)$ to the data for the VAS of mixtures with 15 vol% of glycerol, if assuming that each water molecule interacts with one of adjacent aqueous molecule and one cluster and that the interaction between clusters is neglected.
Figure 5: 3D temporal-frequency plots of apparent VAS for the G/W mixtures with 20 vol%, 33 vol%, 50 vol%, 66 vol% of glycerol
4.2. Rate thickening of molecular friction in G/W mixture

As shown in references [14, 41] the self diffusion coefficient of G/W mixture decreases and the viscosity increases with decreasing temperature according the Stokes-Einstein formula (SE) in a wider range of room temperature. This formula was derived from the Einstein-Smoluchowski (ES) relation [20, 57]

\[ D = \frac{k_B T}{\gamma} \]  

by relating the fluid viscosity \( \eta \) to the friction as \( \gamma = s \pi R \eta \), which is derived from the Navier-Stokes equations for the spherical objects of radius \( R \) moving with small Reynolds numbers in a fluid. Here, the factor \( s \) depends on the boundary condition and is equal to 4 for the slip boundary condition and 6 for the stick boundaries. Thus, the hydrodynamic radius of a molecule \( R \) may be derived directly from the diffusion coefficient using the SE relation, if the viscosity of solution is known. Fig. 7 shows the dependence of water hydrodynamic radius on the glycerol content obtained by using the diffusion coefficients derived from
the MGSE measurement, shown in Fig.8 and the viscosity of G/W mixture from the reference [12] with the assumption of slip boundaries. The values are close to the commonly accepted radius of water, 0.14 nm [78] in the range of higher glycerol content, but slightly smaller at low glycerol content.

When the time scale of the particle is comparable to that of the environmental degrees of freedom, the frictional force felt by the particle will have a memory kernel, meaning that the friction acting on the particle depends on the velocity at an earlier time $\gamma(t)$. This leads to the generalized Langevin equation [34] that gives the VAS in the form

$$D(\omega) = Re \frac{k_B T}{\omega m + \gamma(\omega)},$$

(15)

which can be considered as a generalized ES equation, in the range of low frequencies, $\omega < \gamma(\omega)/m$, or a generalized SE relation, if friction is expressed by a shear rate viscosity $\eta(\omega)$, $\gamma(\omega) = s\pi R\eta(\omega)$. Generalized SE relation was used to obtain the viscoelastic module of a complex fluid from the microscopic motion of small particles [44] in order to understanding the swimming of microorganisms, and the sedimentation in fluids. While the generalized SE gives good estimates for the motion of larger objects, its use for the molecular diffusion could lead to a systematic failure. Such models tend to severely underestimate molecular radius $R$ from the diffusion coefficients or vice versa [22]. Since the hydrodynamic radius of small molecules is weakly dependent on the flexibility and density of the molecular structure, we still can assume the proportionality between the frequency dependent molecular friction and the shear rate viscosity in simple liquids. Thus, the inverse proportionality between the shear rate viscosity and the VAS, $\eta(\omega) \approx D(\omega)^{-1}$, reveals the shear rate thickening of viscosity of our mixtures, which appears at the glycerol contents equal to or larger than 10 vol%.

Our interpretation with the simplified LE confirms that the thickening is a consequence of water interaction with the hydro-cluster formed around glycerol molecules. It corresponds to the common understanding that the shear thickening viscosity is related to the presence of "clumps" in liquids [32].
Figure 7: Hydrodynamic radius of water molecule in the G/W mixture

Figure 8: Diffusion coefficient of G/W mixtures obtained by the MGSE and the PGSE methods [16].
5. Conclusion

On Fig. 8 the diffusion coefficient obtained by the PGSE method as reported in article [16] and obtained here by MGSE method are compared. In MGSE method the VAS at the decay time $\tau = 70$ ms and at the modulation frequency $\nu = 3$ kHz is taken as a diffusion coefficient in order to avoid the part of spectrum with the lumped surface belonging to the diffusion diversity created by the hydrodynamic fluctuations and to be outside the low frequency spectral ridge, since its value cannot be exactly determined due to the low-frequency limit of the method. The results of both measurements match only in the cases of pure water and pure glycerol. The differences may be due to the different interpretation of spin-echo decay, given in Eq. 6 for the PGSE and Eq. 7 for the MGSE method. The article [16] does not mention the width of the MFG pulses at their experiments. This value is crucial for the correct analysis of the PGSE measurements. In addition, the differences may also appear because proportion of glycerol, bounded and unbounded water in the hydroxyl NMR spectral line were not properly estimated in determination of water $D$ [16]. Unlike in the PGSE method, in the processing of the MGSE spin-echo decay no calibration is required to match results of other methods, but one only needs to know the exact value of the MFG as shown in Eq. 7.

However, the study of molecular dynamics in G/W mixture by the NMR MGSE method unveils the low-frequency feature of water VAS, which indicates two: that the small amounts of glycerol in water only partially weakens the hydrogen bonding network in water and thus increases the diffusion rate, and that the glycerol content equal or higher than 10 vol% brings about a new features of VAS, which is the consequence of water interaction with the hydroclusters formed around hydrophilic glycerol molecules. Water-glycerol interaction strongly influence the translational molecular dynamics in liquid resulting in the shear rate thickening of viscosity of the G/W mixture.

The shear rate thickening of viscosity can alter the dynamics of other molecules if immersed in such a liquid. Instead of SE relation, the effect of a liquid on a
submerged molecule one can treat it more properly by the generalized EC equation, which describes the interaction of the liquid with the molecule much more correctly. With that we can speak about the rate thickening of the friction coefficient in liquids, in which a rapid molecular motion and collisions are strongly inhibited. In such G/W mixtures, there are also conditions for the slow process of spontaneously folding of disordered poly-peptides into biologically active protein molecules when immersed in them [27].

As in the cases of MGSE measurements of pure liquids [67], the VAS of G/W mixtures with the low glycerol content show a similarity of the diffusion diversity explained by the molecular self-diffusion in the vortexes of hydrodynamic fluctuation, which disappears at higher glycerol concentrations.

Author contribution statement and acknowledgment

J. Stepišnik, A. Mohorič, C. Mattea, and S. Stapf were all participated in the measurements, the first two in interpretations and preparation of the manuscript, and the other two have read and approved the final manuscript. We acknowledge also the contribution of Prof. I. Serša and Dr. F. Bajd from Josef Stefan Institute to assist in the preparation of experiments on the 100 MHz NMR device.

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