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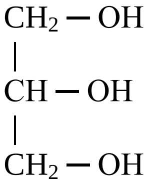
# EXPERIMENT 5A

# Determination of the hydrodynamic radius of molecule by viscosimetric method

**Basic notions:** triglycerides, phospholipids, fats, velocity gradient, dynamic viscosity, liquid viscosity, laminar flow, shearing stress, shear velocity, flow curves, newtonian and non-newtonian liquids, dilating fluids, pseudo-plastic and plastic fluids, hydrodynamic volume and hydrodynamic radius, relative viscosity, light refraction index (refractive index).

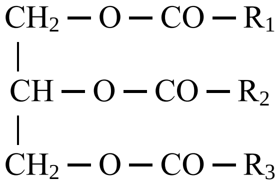
## Introduction

*Triglycerides* are esters of glycerol containing three molecules of fatty acids:



$$\begin{array}{l} CH_2-OH \\ | \\ CH-OH \\ | \\ CH_2-OH \end{array}$$

Glycerol



$$\begin{array}{l} CH_2-O-CO-R_1 \\ | \\ CH-O-CO-R_2 \\ | \\ CH_2-O-CO-R_3 \end{array}$$

Triacylglycerol

Triglycerides are stored in organisms of animals to make a source of energy when needed. They also are structural components of cells, especially nucleus membranes and plasma membranes.

*Phospholipids* are complex fats containing fatty acids and glycerol and phosphoric acid radical. They are main components of biological membranes in which they form a lipid bilayer. The part of the phospholipid molecule containing fatty acids is hydrophobic and thus water insoluble, while the other part composed of glycerol, phosphate and nitrogenous base (e.g. choline) is ionised and easily water soluble. Thanks to this structure, the phospholipid molecules undergo orientation in cell membranes, with their polar parts in one direction and non-polar ones in the opposite direction.

The aim of the experiment is to determine the hydrodynamic radius of the glycerol molecule, making the skeleton of triglycerides and phospholipids. The radius is found from the Einstein's formula for the viscosity of a dispersive system.

## Theory

A consequence of intermolecular attraction in liquids is the resistance exerted by molecules on the motion of the liquid. The force $F$, that should be applied to compensate for the difference in the velocities of two parallel liquid layers of area $S$, separated by a distance d$y$

and moving in the direction $x$, is proportional to the gradient of velocities of these two layers, $\mathrm{d}u_x/\mathrm{d}y$. This relation is expressed by the Newton formula:

$$F = \eta\, S\, \frac{\mathrm{d}u_x}{\mathrm{d}y} \tag{1}$$

where $\eta$ – is the coefficient of dynamic viscosity.

Internal friction, or the liquid viscosity, is observed if the gradient of the liquid velocity is different from zero. The simplest case of internal friction in liquid is the so-called laminar flow of a constant gradient of velocity, perpendicular to the direction of velocity. In this type of flow, the liquid velocity is defined by:

$$u = u_x = y\, \frac{\mathrm{d}u_x}{\mathrm{d}y} \tag{2}$$

$$u_y = u_z = 0 \tag{3}$$

Equation (1) can be converted into the form:

$$F/S = \eta\, \frac{du_x}{dy} \tag{4}$$

where $F/S$ – is the shearing stress. The gradient of velocity $du_x/dy$, is called the shear velocity.

The plots of shearing stress as a function of shear velocity are known as the flow curves. Depending on the character of the flow curve, the liquids are divided into newtonian ones (pure solvents and some diluted solutions) and non-newtonian ones (dispersive systems, colloids and suspensions). The flow curve of a newtonian liquid (ideally viscous liquid) is a straight line passing through the origin of the coordination system. For such liquids, its viscosity is determined by the slope of the linear plot, so the viscosity is independent of the shear velocity. In non-newtonian liquids, viscosity changes with shear velocity and with increasing shear velocity the flow curve deviates towards the y axis (dilating fluids) or towards the x axis (pseudo-plastic fluids). Non-newtonian liquids include the plastic fluids that have the so-called limit of fluidity. The flow curves of such systems do not pass through the origin of the coordinate system.

The simplest model used for the qualitative description of the internal friction phenomenon is the dilute solution of spherical rigid (not undergoing deformation) and non-interacting particles in a solvent of certain viscosity. Such a solution is a Newtonian liquid. In laminar flow, defined by the formulae (2) and (3), different fragments of a given particle in the solution are in the layers of the liquid moving at different velocities. Therefore, the friction forces between the solvent and the solute (particle) cause not only the translational movement of the particle but also its rotation. On the other hand, the particle disturbs the flow of the solvent. Changes in the velocity of the medium do not take place rapidly at the contact of the particle and the solvent, but gradually in a certain layer of the solvent surrounding the particle.

On the basis of the above model and the laws of hydrodynamics, in 1906, Einstein derived the formula for dispersive systems:

$$\eta = \eta_0\, (1 + 2{,}5\Phi) \tag{5}$$

where $\eta$ and $\eta_0$ stand for the viscosity of dilute solution and pure solvent, respectively, while $\Phi$ – is the ratio of the total volume of the dissolved particles to the total volume of the solution. After simple transformations we get:

$$\Phi = N_A \, c \, v \tag{6}$$

where $N_A$ is the Avogadro number, $c$ – molar concentration of the solution and $v$ is the hydrodynamic volume of one particle, so the volume of the particle with the hydration shell.

Deviations from the Einstein formula (5) are observed for solutions of asymmetric particles or flexible particles (undergoing deformation) or for concentrated solutions in which mutual interactions between the dispersed particles cannot be neglected. For such systems, the Einstein equation is modified by introduction of additional terms and corrections. The modified Einstein formula well describes the viscosity of globular protein solutions. As a result of secondary folding of the polypeptide chain and development of ternary structure, globular protein can be approximated as a symmetric macromolecule. The hydrodynamic behaviour of such particles is described by a model of rigid or partly permeable ellipsoids of revolution.

## Experiment

### Method

From equations (5) and (6) it is possible to derive eq. (7) from which the hydrodynamic radius $r$ of a spherical macromolecule can be found:

$$\eta/\eta_0 = 1 + 6{,}3\times10^{24}\, r^3\, c \tag{7}$$

The relative viscosity, $\eta/\eta_0$, of a given solution of macromolecules in this experiment is obtained on the basis of measurement of time of the solution flow through a capillary tube in the Ubbelohde viscometer. In this method, the starting point is the Poiseuille formula:

$$\eta = \frac{\pi R^4 pt}{8VL} \tag{8}$$

where $R$ stands for the capillary tube radius, $p$ – is the hydrostatic pressure of the liquid studied, $t$ – is the time of liquid flow through the capillary tube, $V$ – is the volume of the liquid flowing out of the capillary tube, $L$ – length of the capillary tube.
For the standard liquid of known viscosity, $\eta_w$:

$$\eta_w = \frac{\pi R^4 p_w t_w}{8V_w L} \tag{9}$$

If the volume of the liquid studied is the same as the volume of the reference standard ($V = V_w$), then after division of eqs. (8) and (9) by sides, we get:

$$\eta/\eta_w = \frac{pt}{p_w t_w} \tag{10}$$

Both liquids flow through the capillary tube forced by their weight, so the hydrostatic pressure can be replaced by the densities of the liquids:

$$\eta/\eta_w = \frac{dt}{d_w t_w} \tag{11}$$

In the experiment to be performed the parameter to be found is the effective hydrodynamic radius of a molecule of glycerol. The reference standard is pure solvent (water), so $\eta_w = \eta_0$.

## Reagents and apparatuses

– Concentrated water solution of glycerol,
– Ubbelohde viscometer
– ultrathermostat
– stopper
– analytical balance
– Abbe refractometer
– 6 volumetric flasks of 25 $cm^3$ in volume
– 1 beaker of 25 $cm^3$ in volume
– 1 lab wash bottle

## Procedure

**1.** Light refractive index ($n$) of the initial concentrated water solution of glycerol should be measured with the use of a refractometer. Prior to measurements the surfaces of both prisms of the refractometer should be carefully cleaned with the help of a tissue wetted with acetone, and then dried. Measurements should be repeated 6-7 times, and the refractive index should be read off with the accuracy of four digits after the decimal point.

**2.** In the volumetric flasks of 25 $cm^3$ in volume, you should prepare 6 dilute water solutions of glycerol, by the gravimetric method described below.

Fill a clean and dry small beaker to half volume with the concentrated glycerol solution. Place the first volumetric flask on the analytical balance, take the tare weight, pour from the beaker to the flask the volume of the concentrated glycerol solution corresponding to a mass close to 3.9 grams, put down the mass of the solution poured to the flask with the accuracy to three digits after the decimal point. Proceed in the same way but change the mass of solution poured; the second volumetric flask load with about 3.25 grams, the third with 2.6 grams, the fourth with 1.95 grams, the fifth with 1.3 grams and the sixth with about 0.7 grams of concentrated water solution of glycerol. Each time note carefully the mass of the solution placed in the flask measured to three digits after the decimal point. As follows from the above description, the mass of the concentrated solution of glycerol is measured directly in each volumetric flask, not in the beaker or in any other vessel. Take care that the outside walls of the flasks are dry.

After weighing, the content of volumetric flasks should be supplemented with distilled water up to the mark and carefully shaken.

**3.** The thermostat connected to the Ubbelohde viscometer should be set to 25°C. The viscometer should be filled with distilled water which should be then removed by a water pump, the filling and removal should be repeated a few times.

**4.** The time ($t$) of flow of the solutions studied through the capillary tube in the viscometer should be measured as follows. In the bottom part of one of the viscometer arms there are two horizontal marking bars. The viscometer should be filled with distilled water in such amount that the level of water should be between the two bars. Water should be thermostated for 5 to 10 minutes. With the help of a syringe draw water to the upper container of the viscometer, so that its level was much above the upper marking bar. Release the syringe and measure the time ($t_0$) needed for the water to flow from the upper marking bar to the lower one. Repeat the measurements for 4 or 5 times. The results should be reproducible.

In the same way perform measurements for the 6 solutions of glycerol, starting from the sample of the lowest concentration to the highest one. Each liquid poured into the viscometer should be thermostated.

**5.** Results of measurements write in Table 1.

Table 1

| $c$ [mol/dm$^3$] | $t$ [s] | $t_{śr}$ [s] | $t_{śr}/t_0$ | $d/d_0$ | $\eta/\eta_0$ |
|---|---|---|---|---|---|
| 0,00 | | | | | |
| | | | | | |

## Calculations

**1.** Calculate the mean value of refractive index ($n_{śr}$) of the initial concentrated water solution of glycerol. On the basis of Table 2 data read off the percentage gravimetric concentration of the solution. Calculate the molar concentrations $c$, of the six earlier prepared solutions of glycerol whose viscosity was already measured.

Table 2. Refractive index ($n$) of water solutions of glycerol
(*Poradnik fizykochemiczny*, WNT, Warszawa, 1974)

| % wt | $n$ | % wt | $n$ | % wt | $n$ | % wt | $n$ |
|---|---|---|---|---|---|---|---|
| 71 | 1,4294 | 76 | 1,4368 | 81 | 1,4445 | 86 | 1,4524 |
| 72 | 1,4309 | 77 | 1,4383 | 82 | 1,4461 | 87 | 1,4539 |
| 73 | 1,4324 | 78 | 1,4398 | 83 | 1,4477 | 88 | 1,4554 |
| 74 | 1,4339 | 79 | 1,4414 | 84 | 1,4493 | 89 | 1,4569 |
| 75 | 1,4353 | 80 | 1,4420 | 85 | 1,4509 | 90 | 1,4584 |

**2.** For each sample studied, also for distilled water, calculate the mean value $t_{śr}$, of times $t$ (Table 1), then from equation (11) calculate $\eta/\eta_0$. For water solutions of glycerol it can be assumed that $d/d_0 = 1 + 0.021c$.

**3.** Plot the dependence of $\eta/\eta_0$ on $c$. By the least squares method find the hydrodynamic radius of the glycerol molecule from eq. (7).

## Discussion

1. On the basis of the molar volume of glycerol calculate the radius of its molecule. Compare the calculated value from that determined in the experiment. Interpret the result of this comparison.

2. Prove the validity of eq. (6) and derive eq. (7).

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## Related problems

Measurement of light refractive index; the principle of refractometer operation. Methods of viscosity measurements. Viscosity of macromolecules solutions.

## **Measurement of viscosity with the use of Ubbelohde viscometer**

Measurements of viscosity are performed at 20°C. Between subsequent measurements the viscometer should be washed with the next solution to be studied.

Arm A of the viscometer is loaded with the liquid studied in such amount that its level is between marks 3–3 on arm A. By a water pump the liquid is drawn to the container D, remember that the outlet of arm B should be clogged up. The water pump is released and the fall of the liquid meniscus should be carefully observed, the stopwatch should be started when the meniscus passes bar 1 and stopped it when the meniscus passes bar 2.

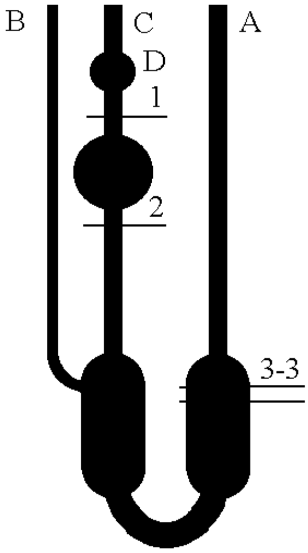
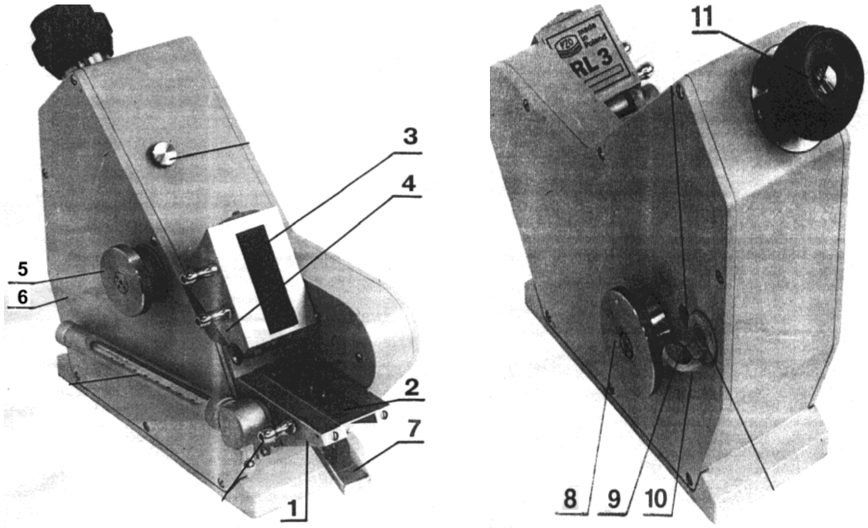


Fig. 1. Ubbelohde viscometer

After completion of measurements the solution should be removed from the viscometer by a water pump. Then, it should be rinsed a few times with distilled water and left filled with water.

## Measurement of light refractive index by a refractometer

Refractive index is measured by a laboratory refractometer RL3, shown in Fig. 2 viewed from two aspects.



a) b)

Fig. 2. Laboratory refractometer RL3 a) a view from the measuring prism and b) a view from the eyepiece.

The main component of the instrument is a refractometric prism in a casing (1) with the horizontally set plane of measurement (2). Above the refractometric prism there is an upper prism (3) which illuminates the substances to be subjected to measurements with the passing light. It is mounted in a hinged frame (4). The mirror (7) is used to illuminate the substance to be measured with the reflected light. During measurement, a beam of light from a natural or artificial source is directed to the refractometric prism through the mirror (7) and the illuminating window in the prism (3). After refraction in the measurement plane the light beam is passed to the inside of the refractometer (6), through the directing prism to the set of Amici prisms. Rotation of the Amici prisms by a knob (5) causes splitting white light. After passing through the set of Amici prisms, the light beam falls onto the objective and is focused in the upper window of the view field of the eyepiece (11).

Fig. 3 presents the view field of the refractometer eyepiece (11). The upper window is divided into the bright (upper) and dark (lower) fields and the borderline between them passes through the point of spider thread crossing. The lower window shows the graduated scale of light refractive indices (it is used in the experiment described above) and the graduated scale of the weight percentage of sugar (the refractometer is used in confectionary industry, pharmaceutical industry and for other applications). The field of view is illuminated with the light directed by the planar mirror (9) mounted in a rotating and swinging frame (10). By rotating the knob (8) the borderline between the bright and dark fields moves in parallel to the horizontal line and at the same time the graduated scale in the lower window moves accordingly.

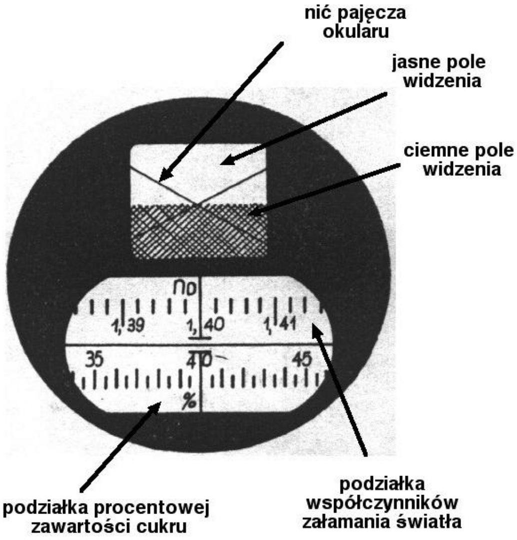


Fig. 3. The two windows in the view field of the refractometer eyepiece.

Measurement

Refractometer prism is illuminated with natural or artificial (a lamp) white scattered light. The procedure of measurement is as follows.

1. Open the shield protecting the window illuminating the prism (3), move away the frame in which the upper prism is mounted (3) till resistance and delicately clean the measurement area (2) and the prism surface (3) with a tissue wetted with ether or other organic solvent.

2. Place a few drops of the substance studied onto the measurement area (2) so that after closure of the prisms the whole measuring area was covered with this substance. Then, lower the upper prism (3) and press it onto the measurement area (2).

3. The window illuminating the upper prism (3) should be directed towards the intensive light source and the mirror (9) should be positioned accordingly.

4. Using the knobs (5) and (8), a clear, sharp division between the bright and dark fields in the upper window should be achieved. Then, using the knob (8) the borderline between these bright and dark fields should be shifted so that is passed through the point of spider thread crossing. When it is done, the vertical line in the lower window of the eyepiece shows the result on the graduated scale of light refractive indices.

*5. After each measurement, the surfaces of the prisms should be carefully cleaned with a tissue wetted with ether or another organic solvent.*

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# Exercise 5B

# Determination of the hydrodynamic radius of a molecule by the viscometric method. Part B. Computer simulations

**Basic notions** Computer simulations, Newton's laws of motion, computer simulations, molecular dynamics, Lennard-Jones potential.

## Introduction

Computer simulations can be divided into two main types: stochastic and deterministic. The first type simulations use random numbers for making a new configuration, while in the second type simulations a new configuration is obtained from the equations of motion. An exemplary method of stochastic simulations is the Monte–Carlo method [1], while an exemplary method of deterministic simulations is the method of molecular dynamics (MD) [2].

Classical method of molecular dynamics is based on numerical solutions of the Newton's equations of motion for a single molecule or for a system of $N$ molecules. The time evolution of the system is most often described by the equation

$$\mathbf{F}_i = m_i\mathbf{a}_i = m_i\frac{d\mathbf{v}_i}{dt} = m_i\frac{d^2\mathbf{r}_i}{dt^2} \tag{1}$$

In which $m_i$, $\mathbf{a}_i$, $\mathbf{v}_i$, $\mathbf{r}_i$ stand for the mass, acceleration, velocity and the molecule coordinates, while $\mathbf{F}_i$ is the force acting on the $i$-th molecule. The forces acting between the molecules can be determined from the potential between the molecules $V_{ij}$

$$\mathbf{F}_i = -\nabla_i V_{ij} \tag{2}$$

where $\nabla$ is the gradient operator. In order to integrate numerically the differential equation (1), the finite and constant time intervals are used, $\Delta t$. Starting from the known values of the molecule position $\mathbf{r}_{0,i}$ and velocity $\mathbf{v}_{0,i}$ at time $t$ , their subsequent values for $t+\Delta t$ are calculated. In computer simulations, one of the most often used methods of integration of eq. (1) is the Verlet algorithm [3].

The function of radial distribution $g(r_{ij})$ [4] defined by equation (3) combines the macroscopic properties of the molecule (bond length, angles, partial charges) with the structure of the system

$$g(r_{ij}) = \frac{N}{V}\frac{n(r_{ij})}{4\pi r_{ij} dr_{ij}} \tag{3}$$

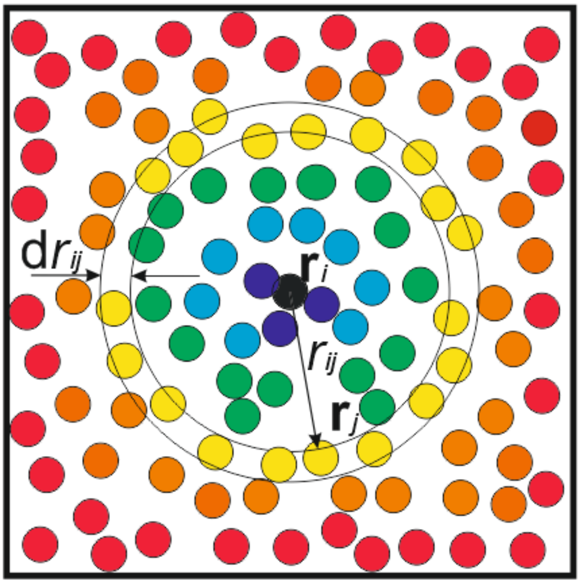
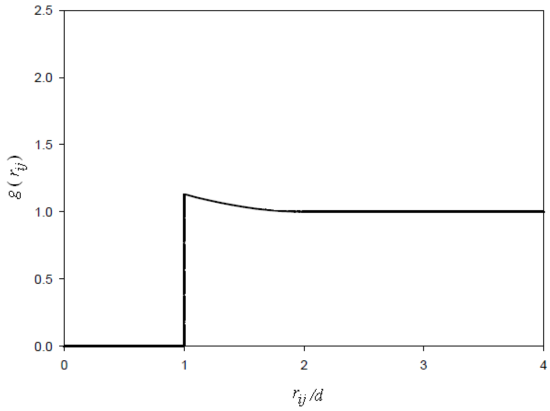
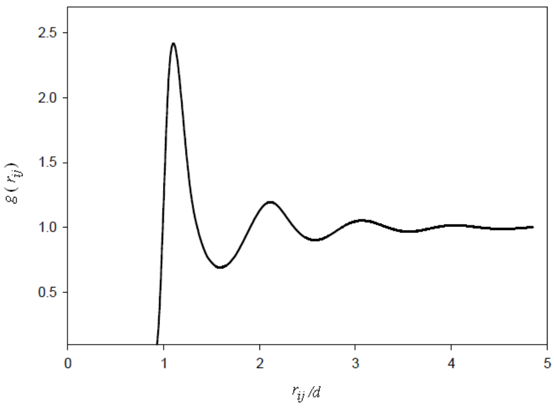


Fig.1 Distribution of molecules in the simulation box

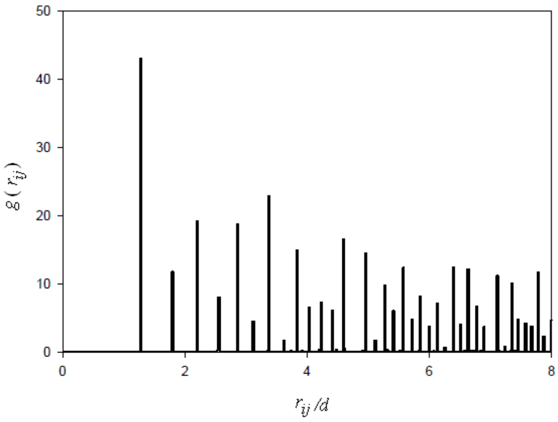
The product $g(r_{ij})\mathrm{d}r_{ij}$ defines the probability of finding a molecule in the infinitely small space element $\mathrm{d}r_{ij}$ at a distance $r_{ij}$. The distance $r_{ij}$ is equal to $|\mathbf{r}_i\text{-}\mathbf{r}_j|$, where $\mathbf{r}_i$ and $\mathbf{r}_j$ are the positions of the central molecule and that of one of the molecules surrounding the central one, $n(r_{ij})$ is the mean number of molecules in the space fragment between $r_{ij}$ and $r_{ij} + \mathrm{d}r_{ij}$, $N$ is the number of molecules and $V$ is the volume of the system [5]. In other words, the function $g(r_{ij})$ defines the distribution of molecules relative to the central one. The radial function can be determined experimentally (by X-ray methods or neutron scattering method), calculated or obtained from computer simulations. Exemplary radial functions for gas, liquid and crystal are as shown in Fig. 2.



a)



b)



c)

Fig. 2. Exemplary functions of radial distribution $g(r_{ij})$ expressed in the units of reduced lengths $r_{ij}/d$ for a) gas, b) liquid and c) crystal.

The dilute systems (Fig. 2a) have molecules uniformly distributed in space, liquids show partial ordering (a few maxima whose intensity decreases with increasing distance from the central molecule) (Fig. 2b). The radial distribution function for a crystal shows a number of sharp maxima, (Fig. 2c), which means that the molecules occupy strictly defined positions in the crystal.

## Practical realisation

### Simulation procedure

The aim of the second part of the exercise is determination of the hydrodynamic radius of glycerine on the basis of the radial distribution function. The radial distribution function is obtained from computer simulations using the packages of Gromacs program [6]. This program has been initially developed for the MD calculations for proteins, fats and nucleic acids, but its subsequent versions permit successful application of the program for the phase interface.

The folder "gliceryna" in the pulpit contains catalogues of data for different concentrations of glycerine solutions. All files needed for the simulation are in this folder. The file `nvt.mdp` contains the parameters needed for the simulation, including the number of configurations (duration of simulation), temperature of simulation, time interval $\Delta t$ , etc. In order to introduce changes in this file and set the simulation parameters, it can be edited in the text editor. All operations related to the simulation are performed through the Linux console. The Linux console is opened by pressing the left key `CTRL` and left key `ALT` and the letter `t`[*]. To enter the catalogue "`gliceryna`" write the following commands:

`cd Desktop` Enter (Caution !!! all commands are confirmed with Enter Enter).

`cd gliceryna` Enter (the contents of the catalogue are displayed in response to the command "`ls`" Enter).

The, you should find the catalogue whose name corresponds to the desired concentration of glycerine solution:

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[*] `only for GNU/LINUX UBUNTU version 12.04`

```
cd c=″ ″ ⏎
```
(*np.* `cd c=1`)

and you should display its contents (`ls`) ⏎.

To see the initial configuration of the system, write the command:

```
vmd glic_NPT.gro ⏎
```

where `vmd` is the program Visual Molecular Dynamics, while `glic_NPT.gro` is the file with the initial configuration of the system. In order to begin the calculation, the following two commands must be written in the console :

```
grompp -f nvt.mdp -c glic_NPT.gro -p topol.top -o output.tpr ⏎
```

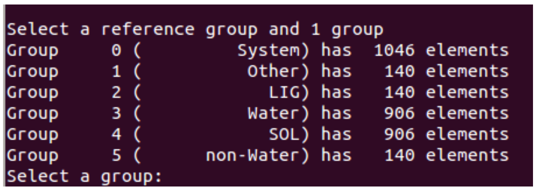
the command `grompp` starts the preprocesor which collects the files with extensions *.mdp, *.gro and *.top to the file *.tpr, the letters `f,c,p,o` stand for the input file, configuration file, topological file and the output file, `nvt.mdp` is the file containing the simulation parameters, `topol.top` is the topological file with the molecule topological data (angles, bonds, partial charges, etc.), `output.tpr` is the name (arbitrary) of the file with the simulation you make. The simulation is started by writing :

```
mdrun -v -deffnm output ⏎
```

After completion of the simulation, you should calculate the radial distribution function, which can be realised by writing the following command:

```
g_rdf -f output.xtc -s output.tpr -rdf mol_com -o rdf.xvg ⏎
```

At the next stage the window of choice appears (Fig. 3), from which we choose a pair of molecules (LIG-SOL) between which the radial distribution function is to be calculated.



```
Select a reference group and 1 group
Group     0 (         System) has  1046 elements
Group     1 (          Other) has   140 elements
Group     2 (            LIG) has   140 elements
Group     3 (          Water) has   906 elements
Group     4 (            SOL) has   906 elements
Group     5 (      non-Water) has   140 elements
Select a group:
```

Fig.3 Exemplary window of choice for the calculation of radial distribution function $g(r_{ij})$

Then you write :

```
2 ⏎

4 ⏎
```

The function $g(r_{ij})$ can be drawn by writing the command

```
xmgrace rdf.xvg ⏎
```

The value of $r_{ij}$, for which the function $g(r_{ij})$ reaches a maximum corresponds to the position at which the probability of finding a pair of molecules is the highest. This distance should be compared with the hydrodynamic radius of the glycerine molecule.

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