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Motion of particles attached to giant vesicles: falling ball viscosimetry and elasticity measurements on lipid membranes

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Abstract

We study the motion of small solid particles (a few micrometers in size) attached to the membrane of a spherical giant vesicle. A set of complementary experiments are carried out on 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in fluid and in gel phases. We provide results on the temperature (T) dependence of the shear surface viscosity, η_S, above the main transition temperature (T_m). A dramatic increase of η_S is observed when T is decreased down to T_m. By means of optical trap manipulation of two particles attached to a vesicle, we stretch/shrink the membrane in gel phase. Following the particles tracks while gradually increasing T allows us to determine the membrane elastic response in the form of an effective stiffness constant. The latter is found to decrease linearly with T. The gel to fluid phase transition is found continuous.

1. Introduction

DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) is a very convenient lipid for studying the thermomechanics of lipid transitions in membranes due to its easily accessible phase transition temperature at ~24°C. Many studies have been carried out aiming at better understanding of bilayer properties when the composing lipid freezes from fluid L_α to rippled gel P_β' phase. Phospholipid phase transitions could be important for example in regulating the activities of membrane-associated proteins [1]. Therefore information on membrane properties in the phase transition region is always substantial in the field. A set of experiments was performed based on the motion of micronsized latex particles in contact with giant DMPC vesicles. This work presents data for the temperature dependence of the shear surface viscosity in the phase transition region of the lipid. The experimental findings well correspond to results provided by the micropipette aspiration technique [2]. Preliminary results on the elasticity of the membrane in the gel phase are obtained by manipulating two particles on a single vesicle by means of optical trapping.

2. Experimental procedures and data analysis

Experiments were performed on giant liposomes prepared from DMPC (Avanti Polar Lipids) by means of the electroformation method [3]. Vesicles (generally larger than 30 μm in diameter) are formed in pure water, in L_α state (usually at 30°C), under electric field (about 1 V, 10 Hz). The investigation chamber (see Fig. 1) is equipped with a circulating water jacket connected to a cryothermostat (Lauda RM6) which allows working at temperatures down to about 14°C. Temperature is controlled by a thermocouple (±0.1°C).

After vesicles are formed, a dilute suspension of latex spheres is gently injected in the chamber away from the cluster of vesicles on the electrode. We use polystyrene spheres

(Polyscience) with radii (a) in the 1÷10 μm range. Beads are manipulated by means of a long working-distance optical trap [4], basically a couple of counter-propagating laser beams (argon ion laser, 514 nm). In bulk water, a trapped sphere equilibrates itself with its center on the laser beam axis. Off-centering the particle by a distance d_{off} produces a linear restoring force (due to radiation pressure) $F = k_{RP}.d_{off}$, provided that d_{off} be small enough, say $d_{off} \leq 0.6a$ [4]. k_{RP} can be regarded as the optical trap spring constant. Typical forces are in the 1÷100 picoNewtons range [4, 5]. No heating of the particles was detected [4]. When a particle is trapped, we first measure its sedimentation velocity in bulk water. Then the particle is brought in contact to a preliminarily chosen vesicle which could be either free or connected to the cluster of surrounding vesicles at the electrode. The nature of particle adhesion and the phenomena observed throughout particle encapsulation into the vesicle membrane is described elsewhere [6]. Generally, spheres stick at the vesicle surface attaining a finite contact angle which depends on the initial tension of the vesicle.

2.1. Viscosimetry (L_α phase)

Already stuck to the membrane, the particle is brought near to the vesicle top and then released to glide down along the vesicle contour till it reaches the bottom. Particle trajectory is viewed from above. The position of the sphere is recorded every 0.2 sec with a resolution of ± 0.5 μm. The friction experienced by the particle sedimenting along the vesicle is influenced by the viscous properties of the membrane. Thus the vesicle/particle system is a realization of a viscosimeter working at the microscopic scale. We studied the temperature dependence of the membrane viscosity near the phase transition region.

The analysis of the particle track from the sedimentation experiment reduces to assessing the friction experienced by the sphere. Particle motion along the vesicle membrane is described by the following equation:

$$\tilde{m}g\sin\theta = \zeta\tilde{R}\dot{\theta} \qquad , \tag{1}$$

where $\tilde{m}$ is the particle mass corrected by buoyancy, g is gravity acceleration, θ is the polar angle as defined in Fig 2. Eq. (1) holds in the "zero-temperature limit", i.e. when Brownian motion is neglected [7]. ζ is the wanted friction coefficient and $\tilde{R}$ is the distance between the particle and vesicle centers. A more detailed analysis on the application of this equation is available elsewhere [7]. To avoid the entropic contribution due to Brownian motion to the measured friction ratio, particles were chosen to be large enough (according to the criterion discussed in [7]). The solution of Eq. (1) reads:

$$f\left[\theta(t)\right] = f\left[\theta_0\right] - \frac{\tilde{m}g}{\tilde{R}\zeta}t \qquad , \tag{2}$$

where $f[\theta] = \text{atanh}\left[\cos(\theta)\right]$, θ_0 defines the particle position at time $t = 0$. Thus the experimentally obtained time dependence of the function f, provides the value of the friction coefficient, ζ. Basically, ζ contains the information about the viscosity of the membrane. In [7], the theory of Danov et al. [8] was used to invert the data about the friction coefficient into shear surface viscosity, η_S, (for 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine bilayers at room temperature, η_S was found to be about $3\ 10^{-6}$ sp). In this work we apply the same approach, [7], to analyze our results. The theory of Danov et al. [8] deals with the motion of a spherical particle along a flat infinite film at the air/water interface. This theory can be adapted to a particle across a membrane (i.e. with water on both sides), provided that a be much smaller than R, the vesicle radius, and that the contact angle be not too far from 90°. When a is not small compared to R, the particle motion induces a circulation of the water

internal to the vesicle. This increases ζ well above the value corresponding to $R/a \to \infty$. An improved theory which takes into consideration this finite-size effect was recently worked out [9].

2.2. Visco-elasticity (gel phase)

Two latex spheres of radii close to 5 μm (beads were chosen to be of similar sizes) are stuck either one by one or simultaneously to a larger vesicle (R > 30 μm), in L_α state. For the optical manipulation we used a double trap configuration of the optical system, i.e. two pairs of laser beams [4,5]. One trap is fixed while the second one can be moved. The distance between the two traps is adjustable between 0 and about 35 μm.

2.2.1. Gel phase static shear elasticity

After particles adhesion, the membrane is cooled down to about 15°C, well in the gel state domain, while holding the two particles at fixed distance, d_0. Afterwards we slowly increase the trap separation to a distance $d_0 + e$. In response to this perturbation, the interparticle distance, d (initially $d = d_0$) increases slightly. The new distance, $d = d_1$, is found < $d_0 + e$ because of the membrane elasticity. Here the membrane can be modelled as a spring of stiffness k_M binding the two particles. The value of d_1 is simply found by balancing radiation pressure (k_{RP}) and membrane (k_M) forces. We find:

$$d_1 - d_0 = \frac{k_{RP}}{2k_M + k_{RP}} e \qquad (3)$$

In our experimental procedure we first measure d_1 at T = 15 °C, then we increase T, which has the consequence of increasing d_1 slightly. The system is left for about 15 minutes to reach equilibrium after each temperature step. We thus build a (k_M, T) graph, up to the main transition temperature, T_m (23 °C ≤ T_m ≤ 24 °C). Afterwards, the system is cooled

again, to repeat the experiment. Thus measuring the interparticle distance for each temperature provides a way to estimate k_M as a function of T.

2.2.2. Gel phase viscosity

A simple extension of the above procedure allows us to study the dynamics of membrane reaction. When the interparticle distance is increased to d_1, the laser beams are switched off. The system then relaxes, i.e. d decreases down to a final value, in principle down to about d_0. In practice we stretch or shrink the membrane by displacing one of the particles (again the double trap configuration is employed). After switching off the mobile trap only, we record the relaxation tracks in time.

If we assume that the particle friction in the gelified system can still be characterized by a constant coefficient, ζ, the above 2-spring model predicts a simple exponential relaxation:

$$d_1 = A + B\exp\left(-\frac{t}{\tau}\right) , \qquad (4)$$

where A and B are constants and $\tau = \zeta/\left(2k_M\right)$ is the characteristic time of the relaxation process. Having already the value of the spring constant, k_M, from the static elasticity experiments for the corresponding temperature, one can estimate approximately the friction coefficient, ζ. Thus the membrane shear viscosity can be assessed even for low temperatures where membrane freezes and sedimentation experiments are not possible.

3. Results

3.1. DMPC L_α phase viscosity : To diminish as much as possible the vesicle finite size effect, we chose to work with systems for which the R/a ratio is large enough - the bead

"feels" the membrane as flat. A set of data for a single vesicle/particle system is shown in Fig. 3. Results are shown in terms of the dimensionless friction ratio, $\bar{\zeta} = \zeta/(6\pi\eta a)$, where η is the viscosity of the surrounding media (bulk water). In Fig. 3, $\bar{\zeta}$ is plotted as a function of the temperature for a particle of radius a = 2.7 μm on a vesicle of radius R = 46.7 μm. The values of η_S, found from those of $\bar{\zeta}$ using Danov et al's theory [7,8], are displayed in Fig. 3 insert, in logarithmic scale. Sedimentation experiments were carried out down to 23°C. Below this temperature, the particles do not sediment anymore (within experimental time, about 1 hour). This was our criterion to decide that the membrane was gelified.

The main feature in Fig. 3 is the considerable continuous increase (3 orders of magnitude) of the viscosity when the phase transition temperature is approached.

3.2. DMPC gel phase elasticity

Statics : the effective stiffness, k_M, is an equilibrium (i.e. static) property of the membrane. The variation of k_M as the temperature is raised is shown in Fig. 4. The absolute value of k_M was estimated from that of k_{RP}, using Eq. (3). We computed the value of k_{RP} by means of the « Generalized Lorenz-Mie » theory (see [5] and references therein), from the characteristics of the particles and knowing the powers of the laser beams in the sample chamber. We thus found $k_{RP} \cong 10^{-3}$ dyne/cm for the conditions used in the experiments.

The different symbols in Fig. 4 correspond to three individual experimental temperature cycles for one and the same vesicle/particles system. Reasonable reproducibility is demonstrated also when the system is altered. Although preliminary, these results show an approximately linear variation of k_M in the studied temperature interval. When the lipid is in L_α phase the bilayer looses its elastic properties, the membrane becomes fluid and $k_M = 0$.

The dispersion of the results and the experimental error for lower temperatures increase because the membrane becomes stiffer and particles displacement is smaller.

In principle, it should be possible to extract the value of the membrane shear modulus, μ, from that of k_M. A simple limit is that of a plane infinite membrane, with 2 spheres whose centers are located in the membrane plane (this corresponds to 90° contact angles for both particles). In this case, and using Muskhelichvili's analysis for the elasticity of 2-dimensional systems [10], we predict :

$$k_M \cong C.\mu \tag{5}$$

Here C is a constant $= 8\pi/\ln(d_0/a)$. This results holds if $d_0 << a$ and if the membrane compression modulus K, is much larger than μ (for DMPC bilayers, K is at least on the order of 100 dyn/cm). In our conditions $d_0/a \approx 5$.

If we directly apply Eq. (5) to our data, we find $\mu \approx 10^{-4}$ dyn/cm at the lower end of the investigated temperature range. Notice that such a small value of μ is a posteriori consistent with the above mentioned $\mu << K$ condition. The estimated value of μ is however surprisingly small for a supposedly ordered phase [11]. In fact, k_M could be considerably smaller than $C\mu$, because the particles in our experiments were almost completely inside the vesicle ; in other words, the contact angles were very small. Pulling the particles apart by means of the laser beams most probably makes them roll and induces an out-of-plane (more precisely out-of-sphere) deformation of the membrane. This effect needs to be taken into account for the value of μ to be correctly estimated. In any case, the above mentioned value should be taken as a gross lower boundary.

Dynamics : We studied the membrane relaxation dynamics following the protocol explained in 2.2.2. An example of interparticle distance relaxation is shown in Fig. 5. The data is nicely

fitted to by a simple exponential function (solid line), with $\tau \cong 5$ sec. With k_M =1.3 10^{-3} dyn/cm, the value at T = 19°C, we find $\bar{\zeta} \approx 140$. Such a friction corresponds to about 0.01 sp in a fluid membrane, i.e. about 3000 times the viscosity of DMPC well above the main transition.

4. Discussion and conclusions

The approach presented in this work gives an overview on both viscous and elastic properties of vesicle membranes when the phase transition region is crossed. In summary, three types of experiments were performed. Following the particle track in the sedimentation experiments provides a direct measurement of the membrane shear viscosity, η_S. Unlike techniques as Fluorescence Recovery after Photobleaching, Electron Spin Resonance, or Nuclear Magnetic Resonance, which are sensitive to molecular parameters, our method works on macroscopic scale, therefore not requiring a theory relating molecular dynamics to macroscopic properties. The sedimentation experiments demonstrate a gradual change in the properties when the phase transition temperature is approached, which is the indication of strong pretransitional effects.

To our knowledge, we provide the first measurements of a lipid bilayer linear elastic response, i.e. the basic macroscopic characteristic of a solid structure. We found that the membrane effective stiffness decreases down to zero (within experimental error) at T_m, which is the indication of a continuous gel-fluid phase transition.

These results are in line with others obtained by different -but less direct- techniques on unilamellar systems. For instance, ultrasound propagation and attenuation were found continuous at T_m [12] and a critical slowing down of membrane relaxation was observed [13]. Pretransitional phenomena in L_α phases near T_m are correlated to structure fluctuations in the

form of gel-like domains, of finite size and lifetime and whose existence was experimentally detected by fluorescence spectroscopy[14].

Acknowledgements

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Figures captions

Fig. 1 : Scheme of the temperature controlled chamber. The sample is contained inside the central area (size $\cong 40 \times 9 \times 1$ mm^3). This zone contains two platinium wires (0.8 mm in diameter) for vesicles electroformation, a syringe needle for water and solid particles injection, and a thermocouple (not represented). The zone in light grey is the volume flooded by the circulating water. The sample and flooded zones are bounded by microscope cover slips (170 μm in thickness). The thickness of the chamber at the level of the symmetry axis is $\cong 3.4$ mm. With the microscope objectives used in the set-up (Zeiss Epiplan, $\times 50$, N.A.=0.5), the entirety of the sample volume is accessible to observation and optical manipulation.

Fig. 2 : Sedimentation experiment. Illustrative sketch of a particle attached to a spherical vesicle and bead trajectory when falling along the vesicle membrane. See text for definitions of symbols.

Fig. 3 : Dimensionless friction ratio, $\bar{\zeta}$, as a function of the temperature for a single vesicle/particle system. The insert presents the same data inverted to shear surface viscosity (η_S).

Fig. 4 : Static elastic experiments data. Membrane effective stiffness constant, k_M, plotted as a function of temperature. Different symbols correspond to three individual temperature runs.

Fig. 5 : Dynamic elasticity experiment at $T = 19$°C. The points represent the measured interparticle distance, d_1. The solid line is an exponential fit for the relaxation part of the experiment according Eq. (4), $\tau = 5$ sec.

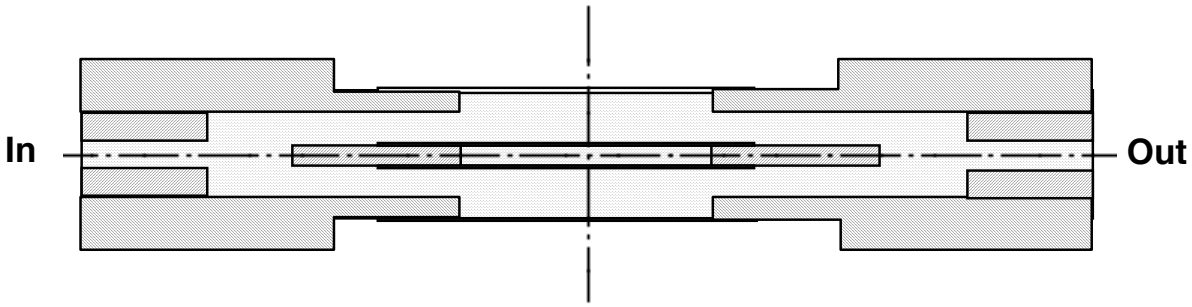


Fig. 1

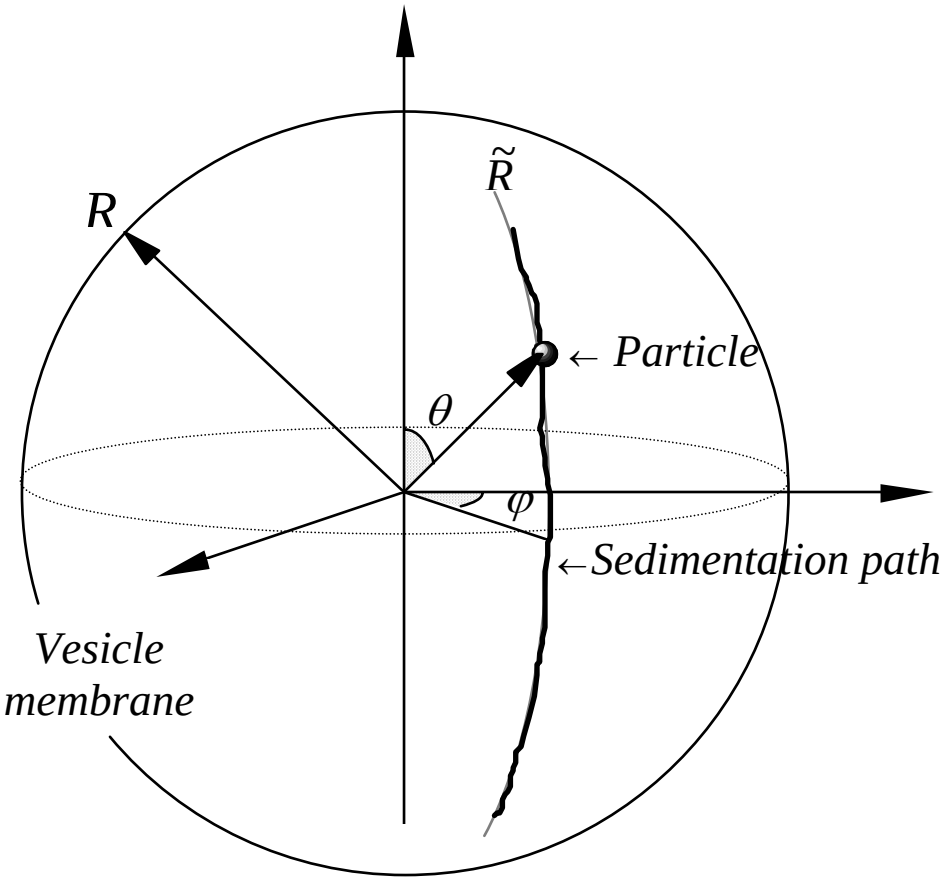
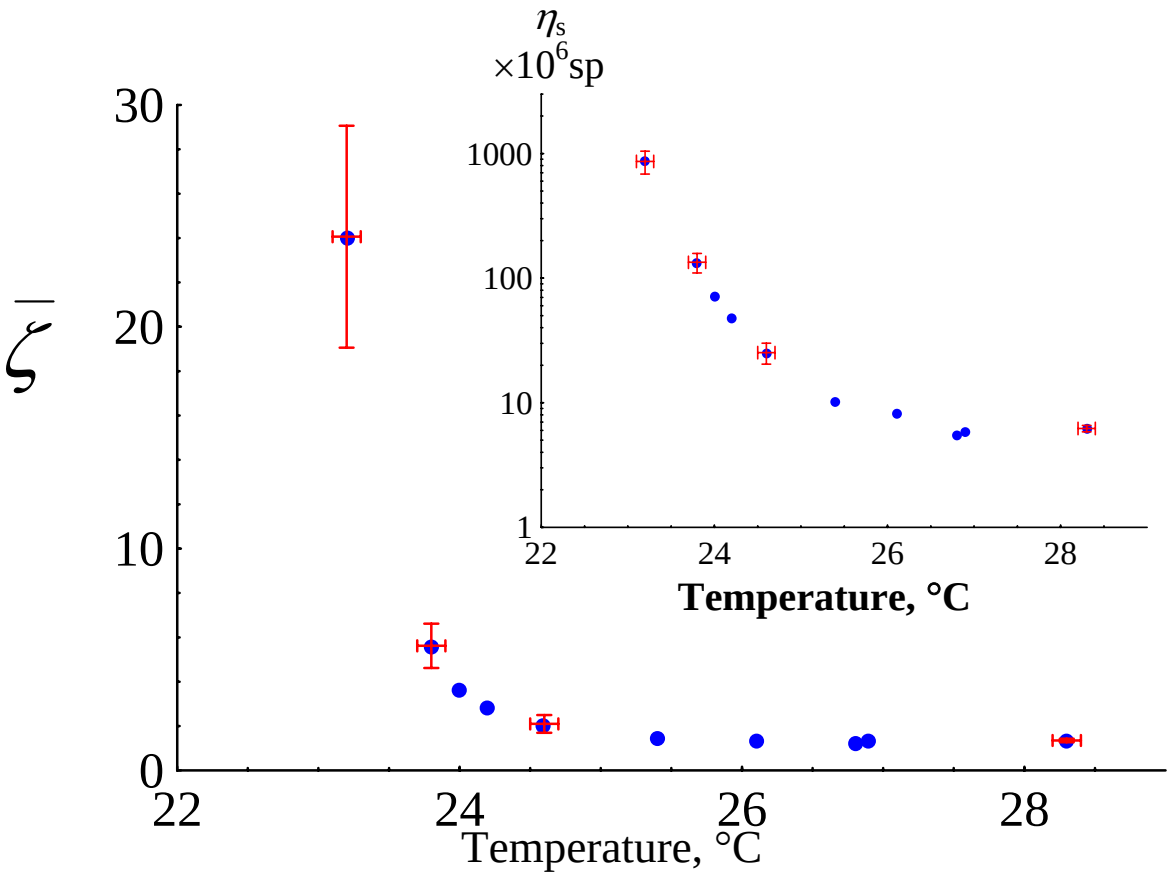


Fig. 2


η_s
$\times 10^6$sp
1000
100
10
1
22
24
26
28
Temperature, °C
$\overline{\zeta}$
30
20
10
0
22
24
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28
Temperature, °C

Fig. 3

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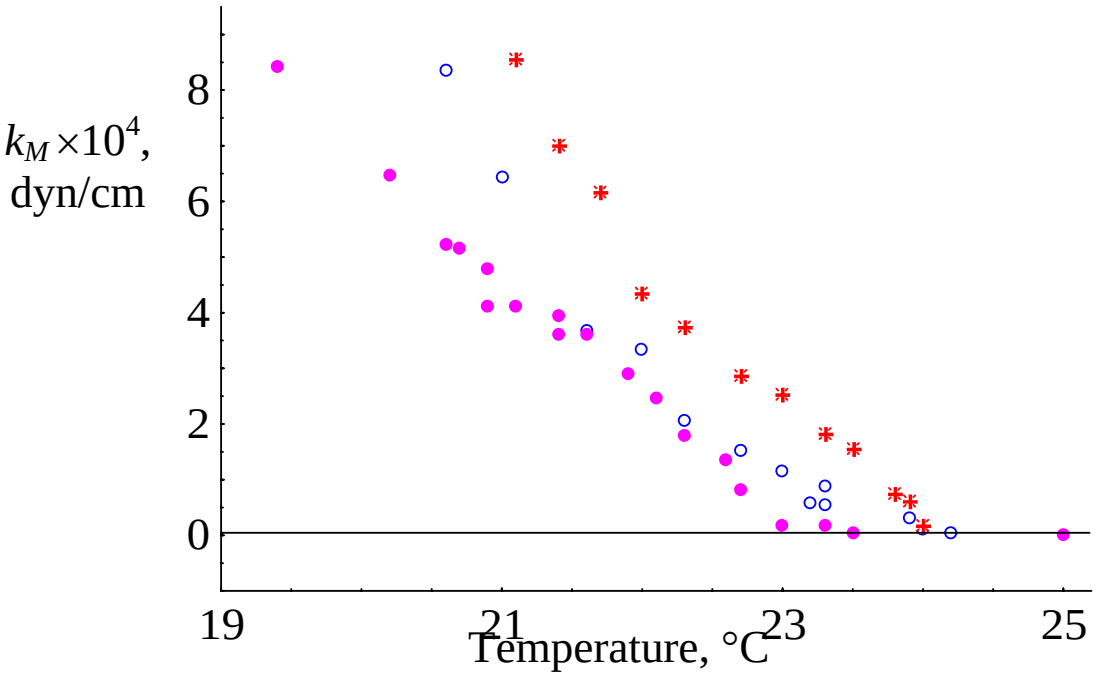

Fig. 4

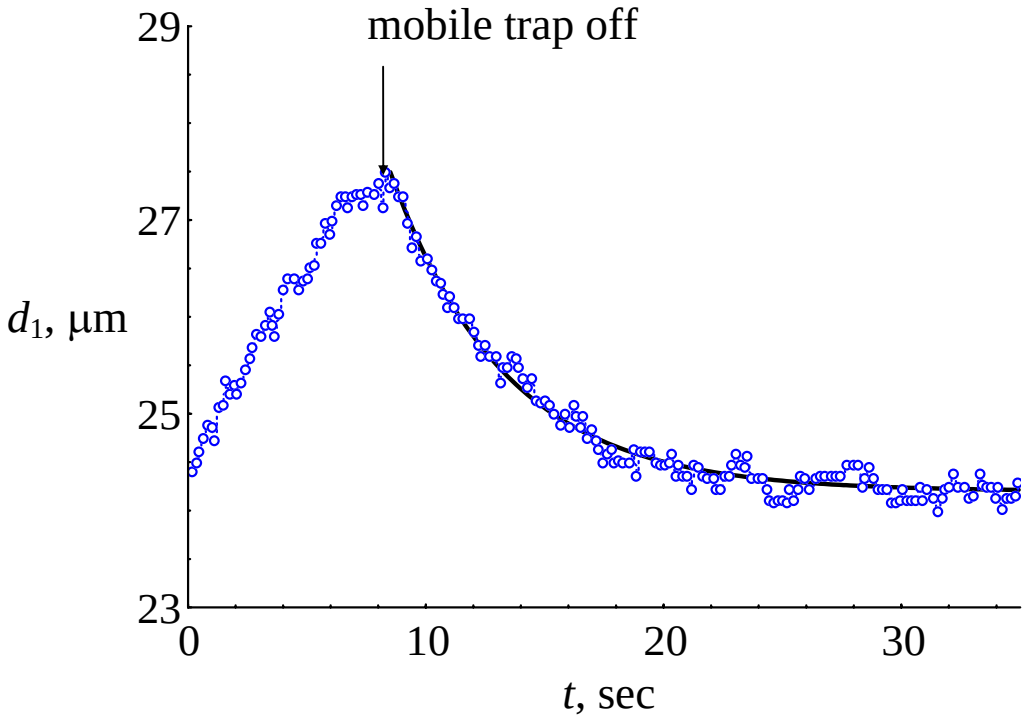

Fig. 5