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The theory of membrane "vitrification" [1]

M.V. Voinova

Department of Theoretical Physics, Faculty of Physics, Kharkov State University, Svobody Sq., 4, Kharkov 310077, Ukraine

Abstract

This review covers the application of the Onsager fluctuation theory and reciprocal principle to nonequilibrium membrane phase transitions (PT) with diffusion. The main features of membrane "vitrification" theory are discussed for spherical lipid membranes subjected to the fast cooling. The relevance to cryoprotection problems is discussed.

Keywords: Cryoprotectors; Fluid lipid membranes; Membrane vitrification; Nonequilibrium phase transitions; Onsages fluctuation theory

1. Introduction

Although the thermodynamic nature of the glass transition (GT) and the glassy state was declared as well understood more than thirty years ago (for the early thermodynamics of GT see, e.g., Refs. [1–4]) the construction of the generalized molecular theory of the GT and supercooled liquid dynamics remains to be completed [5–8]. There is also a gap between the modern statistical mechanics of glassy states and dynamic approaches. Overcoming these theoretical problems seems to be important.

In recent years much experimental and theoretical effort has concentrated on the liquid–glass transition. Thermodynamic and kinetic theories including energy landscape description or percolation models are widely used for the explanation of this phenomenon [6, 8].

Dynamic approaches treat the relaxation of glassy states as a system containing a large number of highly interacting particles. The major progress in the dynamic analysis of the liquid–glass transition is supported by advances in mode coupling

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[1] Dedicated to Professor Hiroshi Suga.

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theories (MCT; for reviews see Refs. [8–10]). Since a liquid–glass transition is an example of a nonequilibrium phase transition, the correlative functions and their derivatives determine the transferring coefficients and suffer jump-like changes [11]. The main concept of MCT is the nonlinear coupling between pairs of density fluctuations. Some MCT predictions have recently been verified in polymeric liquids [12]. Another simple system which shows a dynamic transition is a colloid of hard spherical particles. In accordance with MCT results [11], increasing the colloid density would lead to increased coupling strength and the appearance of a dynamic transition at some critical density (or critical temperature, T). The interaction has been introduced as a function of the hard sphere diameter which determined a microscopic length scale. Temperature did not enter to this definition, setting the time scale only [11]. Recently the latter system has been investigated experimentally in a supercooled suspension of spherical colloidal particles of nearly equal sizes which form dense fluid-like, crystalline, and glassy (amorphous) states [13]. When the concentration of such a colloid was increased, the classical GT scenario was demonstrated [13].

We would emphasize that the afore-mentioned results may play a key role in the study of the glassy state stability of high-molecular weight substances called cryoprotectors which are used for the protection of living cells or liposome emulsions against crystallization damage during freezing–thawing (see also the last section of this paper).

Modern dynamic theory of liquid–glass transitions uses an apparatus of correlation functions which is deeply connected with fluctuation dissipative relations and the Onsager reciprocal principle [14,15]. The purpose of this paper is to combine the Onsager fluctuation theory and coupled transport problems with the thermodynamic and kinetic features of nonequilibrium phase transitions in fluid lipid membranes.

The glass-like behavior of synthetic membranes (referred to here as "membrane vitrification") on rapid cooling has recently been reported as a 2D analogy of the bulk glass transition in commonly used glass formers [16]. Artifical lipid membranes are typical smectic liquid crystal systems [17] which are useful experimental models for studies of phase transitions in low-dimensional systems [18]. In contrast with the Langmuir–Blodgett technique, the most easily prepared aqueous colloids of spherical lipid particles (micelles or liposomes) present the possibility of investigating curvature effects on a mesoscopic scale [19].

2. Fluctuations in Onsager theory and coupled transport processes

Let us consider the extensive variables x_i characterizing our system. The linear relaxation of mean values $\overline{x_i}$ to their equilibrium states is described by the Onsager principle [14, 15]:

$$\frac{\mathrm{d}\overline{x_i}}{\mathrm{d}t} = \sum_k B_{ik}\overline{x_k}; \tag{1}$$

$$B_{ik} = \sum_m L_{im} S_{mk};$$

$$S_{mk} = \left(\frac{\partial^2 S}{\partial a_m \partial a_k} \right)_e;$$

$$\bar{a} = \overline{x - x_e} = \bar{a}_0; \quad \overline{a(0)} = a_0.$$

Here and subsequently S is the system entropy, B is a relaxation matrix, and subscripts 0 and e denote the original and equilibrium states, respectively. The mean thermodynamic flux of variable x_i is defined as the time derivative of $\overline{a_i}$:

$$\overline{J_l} \equiv \frac{\mathrm{d}\overline{a_l}}{\mathrm{d}t} = \sum_m L_{lm} \overline{X_m}, \tag{2}$$

$$X_m = \frac{\partial S}{\partial x_m} - \left(\frac{S}{\partial x_m} \right)_e$$

where X_m denotes the generalized thermodynamic forces which cause the relaxation to the equilibrium, and cross coefficients satisfy the Onsager reciprocal relationship $L_{lm} = L_{ml}$. In the matrix from the kinetic equation is

$$\frac{\mathrm{d}\bar{a}}{\mathrm{d}t} = B\bar{a} \tag{3}$$

and the solution is $\bar{a}(a, t) = \exp(Bt)a_0$, where a and a_0 are matrices [15].

The nonequilibrium statistical ensemble case, the Onsager principle, Eq. (3) can be generalized [15]

$$\frac{\delta a}{\mathrm{d}t} = B\delta a + \tilde{y} \tag{4}$$

where $\tilde{y}$ is a stochastic term, so for $t > 0$ the vector deviation $\delta a(t)$ is nonzero and would satisfy a dynamic equation resembling a Langevin equation for Brownian motion.

The basic relationships of the Onsager principle, Eqs. (1)–(3) are naturally connected with open systems analysis by mean of irreversible thermodynamics [20]. The well known illustration is the phenomenological description of coupled transport processes [21], in particular ionic fluxes or the diffusion of neutral components through semipermeable membranes. The phenomenological relationships in Kedem–Katchalsky theory combine the corresponding fluxes and generalized forces. In the linear approximation

$$J_V = L_{11} \Delta p + L_{12} \Delta \Pi, \tag{5}$$

$$J_\mathrm{d} = L_{21} \Delta p + L_{22} \Delta \Pi, \tag{6}$$

$$J_V = J_\mathrm{w} + J_\mathrm{d}. \tag{7}$$

Here the volume flux J_V is the sum of water flux J_W and interchange flux J_d which corresponds to the relative displacement of diffusing substances and water molecules through the membrane; the diffusional cross coefficients satisfy the Onsager reciprocal

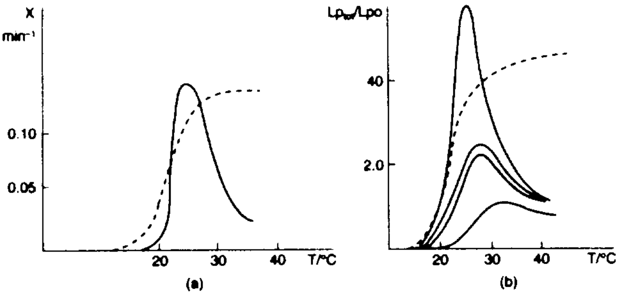

Fig. 1. Temperature-dependence of DMPC (dimyristoyl phosphatidylchdine) vesicles: (a) Experimental data from Ref. [23]. The solid line is the initial rate of uptake X of tempo-choline into DMPC vesicles. The dashed line is the limiting uptake after a long period. (b) Calculated dependence of the resulting water permeability. The solid lines correspond to results from Ref. [33]. The dashed line is slow membrane response in the PT region recalculated from experimental data (see Ref. ([15]) in Ref. [16]).

relationship $L_{12} = L_{21}$. For ideal semipermeable membranes $L_{11} = -L_{12} = L_{22}$, so

$$J_V = L_{11}(\Delta p - \Delta \Pi_{\text{osm}}), \tag{8}$$

where the osmotic pressure difference $\Delta \Pi_{\text{osm}} = RT\Delta C$, R is the universal gas constant, T is Kelvin temperature, and L_{11} is a coefficient of water filtration. Eq. (8) in its most general form is widely used for the description of passive membrane transport [22].

Drastic changes in membrane permeability for ions and small neutral compounds occur in the vicinity of the membrane phase transition [23–28] (Figs. 1a and 1b). The following sections of this paper contain the application of the Onsager principle, Eq. (4), to the nonequilibrium membrane phase transition accompanied by diffusion.

3. Membrane fluidity and stability of pore formation on cooling

Originally the problem of the stability of spherical membranes undergoing rapid pressure and temperature perturbation on rapid cooling arose from thermal shock experiments on living cells [29]. It has been shown that the process of cell lysis is dependent on the cooling rate and osmotic conditions [30, 31]. The most dramatic events occur at subzero temperatures, when cooperative structural transitions of proteins and the lipid membrane matrix take place. Modern experimental studies of lipid dispersion phase transitions (PT) have demonstrated the nonequilibrium and nonisothermal nature of these transitions. So, a finite PT temperature width and dependence of the calorimetric peaks on the rate of change of temperature have been reported elsewhere [32, 33]. Thus, it is an real problem to construct a theory relating phase transition kinetics to membrane stability, i.e. the dependence of its elasticity and permeability on thermal and osmotic gradients [33].

Closed lipid membranes (liposomes or vesicles) may be considered as a special case of thin elastic shells. Accordingly, their elastic properties enable a continuum-mechanics description. It is well established [34–37] that a lipid bilayer membrane is deformable in a more than one way. Lipid membranes are often regarded as two-dimensional liquids. Membrane fluidity is observed above the main transition region where the hydrocarbon chains are in the molten (disordered) state. In fact, membrane fluidity is a consequence of vanishing shear modulus. In the absence of stretch deformations, the shape of such a fluid vesicle is controlled by the membrane curvature elasticity [34–37]. The total elastic energy of fluid membrane deformation is

$$E = \frac{D}{2}\int (K - K_0)^2 \mathrm{d}A + \frac{\Gamma}{2}\int \left(\frac{\delta A}{A}\right)^2 \mathrm{d}A. \tag{9}$$

Here the first term corresponds to the elastic energy of bilayer bending; K is the mean membrane curvature, and K_0 is the spontaneous curvature introduced by Helfrich [35]. The second term is the elastic energy of membrane stretching; D and Γ are the bending modulus and elastic modulus of membrane stretching, respectively. For spherical membrane surface stretching, the excess surface pressure is equal to the Laplace one:

$$\Delta P = \frac{2\Pi(A)}{r}, \tag{10}$$

where r is the mean spherical membrane radius and $\Pi(A)$ is the surface (lateral) pressure which depends on the membrane area per lipid molecule:

$$\Pi = -\left(\frac{\partial \mu_c}{\partial \alpha}\right)_T \tag{11}$$

$$\alpha = (a - a_0)/a_0$$

where the free energy of hydrocarbon chains per molecule, μ_c, corresponds to the sum of gauche and *trans* conformations. The extensive variable surface area A and the conjugate surface $\Pi(A)$ are introduced as the 2D analogy of the common thermodynamic P–V variables. There are $\Pi(A)$ variables which are involved in fluid–solid membrane isobaric phase transitions [26].

In recent years major progress has been achieved in experimental and theoretical studies of phase transitions in synthetic lipid emulsions (see, for reviews, Refs. [38–45]). As an example, a high temperature PT in the lipid DPPC (1,2 palmitoyl-sn-glycerd-3-phosphatidylcholine) from a fluid (f) phase in which the hydrocarbon chains are disordered through a ripple phase in which the chains are more ordered but tilted (pre-transition) to a solid (s) gel phase where the chains are in all-*trans* state, occurs in the region 41.4–34°C. When the temperature is reduced below this interval, a subtransition appears at 13.8°C from the gel phase to a more crystalline one where the positional ordering of the chains is much greater [18]. A main phase transition is characterized by significant changes in the surface area per molecule [32, 46]:

$$\kappa = \frac{a_\mathrm{f} - a_\mathrm{s}}{a_\mathrm{f}} \sim 25\text{–}30\%. \tag{12}$$

Such essential lateral diminishing is accompanied by chain elongation so the molecular volume reduction is not large [18]. Let us introduce a 2D equilibrium order parameter corresponding to the original phase concentration. Starting with the fluid phase state

$$\Theta_e = N_f/N_0. \tag{13}$$

The conventional $\Theta(T)$ dependence is a sigmoidal function which may be obtained from slow (isothermal) experimental data or approximated by the two-parameter Avrami curve [18, 47]. On fast cooling the decrease in the concentration of fluid phase produces excess surface pressure in the closed membrane when the water flow has no time to compensate for the surface area changes. Above a threshould value, lateral tension can lead to rupture of the membrane and the formation of a macroscopic hydrophilic pore [48–54]. The critical tension for the bilayer vesicle can be obtained by minimizing the elastic energy of a stretched membrane with a pore [50]:

$$E_m = \Gamma A \left(\frac{\Delta A}{A} - \frac{\pi\rho^2}{A}\right)^2 + 2\pi\rho\gamma. \tag{14}$$

Here γ is the linear pore edge tension and ρ is the pore radius. This critical tension corresponds to the threshould magnitude of the fluid phase concentration [55]

$$\Theta_c = 1 - \frac{3}{2\kappa}\left(\frac{\gamma}{2\Gamma r_0}\right)^{2/3}. \tag{15}$$

From Eq. (15) it follows that membrane rupture would occur at the very beginning of the PT region. The kinetics of pore nucleation and growth should, however, be considered under highly nonequilibrium conditions [54, 56].

4. Membrane “vitrification”

It is generally accepted that the kinetics of lipid phase transitions are studied by measuring the vesicle volume as a function of time when the temperature is lowered [18, 42]. The “temperature-jump” method or dilatometrical “quenching-mode” technique [18] makes it possible to obtain the relaxation volume response to a sharp temperature perturbation. There are, however, different relaxation times in the water-permeable elastic vesicles [16, 33]. Thus, it is important to develop a model describing the kinetics of volume and permeability of a vesicle for an arbitrary cooling rate.

We have previously considered intermediate cooling rates [33]; the relationship between membrane tension, water flow through the membrane, and the phase state of lipids was determined. In this section we give a short overview of results obtained by rapid cooling, including the possibility of glass-like membrane behavior reported earlier [16].

In the approximation of linear coupling between the generalized forces and flows, the change in the liposome volume due to the water loss is described by the relationship:

$$J_V = \frac{d(V - \tilde{V})}{dt} = L_P(T)(A_f - A_h)(\Delta P_L - \Delta\Pi_{osm}), \tag{16}$$

L_P is the membrane filterability to water which depends on temperature, A_h is that part of the membrane surface area which is impenetrable to water [33]. The equation of state connects the temperature varying Laplace pressure and the lateral tension:

$$\Delta P_L \tilde{r} = \Gamma(A - \tilde{A})/\tilde{A};$$
$$\tilde{A} = \tilde{A}_f + \tilde{A}_s;$$
$$\tilde{A}_f = A_0 \Theta(T).$$

Within the model considered, the initial state is completely fluid and the tilde denotes the unstrained values. The temperature-dependence of the concentration of unstrained fluid phase may be recalculated from the isothermal (slow) experimental data (see Fig. 2). But if the temperature of cooling changes very quickly with time, the unusual relaxation scenario of the PT may be realized.

Following Refs. [18] and [33], we present the 2D order parameter in the form:

$$\Theta_c = 0.5\{1 + (G-1)/[(G-1)^2 + 4\sigma G]^{1/2}\} \qquad (17)$$
$$G = \exp[\Delta H_t(1 - T_c/T)/RT_c].$$

Here ΔH_t is the enthalpy change, σ is the cooperativity parameter, and T_c is the transition temperature determined at the inflection point of the sigmoidal $\Theta(T)$ curve. Although Eq. (17) has been obtained for the one-dimensional transition in a linear chained molecule [23], this function well describes the experimental data at the beginning of the PT (Fig. 2a).

Let us consider the solidification of the membrane under nonequilibrium conditions. We describe the membrane relaxation processes under cooling on the basis of the kinetic equation for a first order chemical reaction:

$$\mathrm{d}\Theta/\mathrm{d}t = -(\Theta - \tilde{\Theta}_c)_{\tau^{-1}} \qquad (18)$$

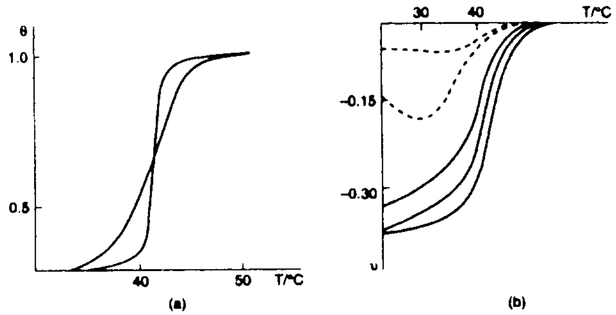

Fig. 2. (a) Calculated temperature-dependence of the order parameter of DPPC vesicles at various cooperativity parameters from Ref. [33]. (b) Calculated dependence of DPPC vesicles volume for various cooling rates and k values from [16]. The dashed line corresponds to the glass-like behavior predicted.

The inverse relaxation time of the bilayer τ is equal to the sum of probabilities of the transition from fluid to solid state and vice versa. When this τ value is not dependent on time, simple exponential relaxation is realized:

$$\Theta = \Theta_e[1 + \exp(-t/\tau)]. \tag{19}$$

Let the temperature be a linear function of time. In this case it is possible to choose the ratio between the rate of cooling q and the temperature derivative of the relaxation time $\mathrm{d}\tau/\mathrm{d}T$ at which the transition is halted:

$$|\mathrm{d}\tau/\mathrm{d}T|_{T_g} = 1, \quad \mathrm{d}T = -q\mathrm{d}t. \tag{20}$$

This condition was first found in Ref. [2] as a criterion for the vitrification of bulk liquids, and T_g has been classified as a temperature of glass transition. Starting with temperature T_g a new (solid) phase is no longer produced in the time scale of numerical simulations. In fact, we found a two-dimensional analogy of the latter transition for the bilayer membrane. The originally fluid membrane, when undercooled in the transition region, would solidify to a quasi solid-state gel (or subgel) phase with formation of macroscopic domains, or would form a glass-like amorphous state if "crystallization" did not intervene [16].

Let us consider the role of tension in the predicted glass-like transition for spherical unilammelar vesicles. First, we suggest the T_c value may be shifted by the varying Laplace pressure, which in its turn depends on the rate of cooling. We introduce this shift as a fictive temperature T_c^* [33]:

$$(T - T_c^*)/T_c = k\Delta P(q). \tag{21}$$

Here the proportionality coefficient k is our model parameter.

It is possible to represent Eq. (16) in a more compact form:

$$\frac{\mathrm{d}v}{\mathrm{d}t} \approx \frac{1}{V_0}\left[\frac{a - \tilde{A}}{16\pi\tilde{r}}\frac{\mathrm{d}\tilde{A}}{\mathrm{d}t} + \frac{\tilde{r}}{2}\frac{\mathrm{d}(A - \tilde{A})}{\mathrm{d}t}\right]; \quad \frac{\mathrm{d}A}{\mathrm{d}t} = -\frac{A - A_e}{\tau}, \tag{22}$$
$$v \equiv \Delta V/V_0 = r(A - \tilde{A})/2V_0.$$

where the unstretched surface area $\tilde{A}$, the vesicle radius, $\tilde{r}$, and the relaxation time of the system, τ, are the cumbersome functions of the original membrane state, its geometric size, elastic properties, permeability to water, and of the rate of cooling [56]. The substitution of Eqs. (17)–(21) in Eq. (16) gives a set of self-consistent equations for the enclosed volume kinetics. These equations have been solved numerically for the lipid DPPC (Fig. 2b). From the results presented in Fig. 2b it follows that the small k values only shift volume curves. Starting with threshould k_c value, however, the volume kinetics slow down sharply. Below the T_g temperature the enclosed volume hardly changes. This point we identify as the previously mentioned glass-like membrane transition. The case of zero k value corresponds to the tension-independent ("unstretched") phase transition. In such a situation the glass-like state may be realised far from the main transition region. Besides, it is shown that in our model the increase in k value is equivalent to the increase in cooling rate. A similar result is obtained by decreasing the value of L_P. Apparently, the coincidence of these results is a consequence of linear coupling between the generalized forces and flows (Eqs. (1–3)) used in our model.

5. Spectrum of relaxation times and dependence of the effective transition temperature on cooling rate

The phase transition temperature T_c in Eq. (17) can be defined as an inflection point of the conventional kinetics or equilibrium sigmoidal Θ curve. It is well known that the shift in the T_c temperature can be caused by application of additional pressure [32]. Also, the rapid compression of monolayers by the moving barrier leads to the formation of dendrides rather than circular domains [57]. In our analysis we have introduced the effective temperature T_c^* as a function of the lateral pressure $\Pi(A)$ or surface tension α, deriving this pressure-dependence as a Taylor expansion of a series of small α values:

$$T_c^* = T_c(1 + k_1\alpha + k_2\alpha^2 + \ldots). \tag{23}$$

The quadratic α term corresponds to the existence of elastic energy of stretching in the form of Eq. (9). The linear term in the expansion arises from the contributions of fluid phase and phase boundary to the transition enthalpy. Previous section of the paper, introduced only linear coupling between the effective transition temperature and surface area changes. Nonlinearity appears only as a consequence of application of the Onsager principle, Eq. (4). Near the transition point, a combination of the oppositely directed processes of surface membrane tension and water outflow, which depends on the phase state, cause slowing of the volume kinetics in the PT region when cooling is sufficiently fast. In our analysis the effective transition temperature T_c^* is introduced only as an implicit cooling rate function. This T_c^* value is rather different from the fictive temperature of glass, T_f. The latter is defined qualitatively as the temperature at which the equilibrium liquid has a relaxation value of the system macroscopic property the same as that of the system in a referred state [58, 59]. Next, T_f values which have been defined for the same state assessed from different properties were not necessarily identical [58].

Isothermal structural relaxation may be described phenomenologically in terms of the evolution of independent n_i order parameters [57]. A large number of theoretical studies define a relaxation time associated with each order parameter in rather different forms. By way of illustration, one widely used definition is [57]

$$\tau_1 = a_1 \exp\left\{\frac{b\Delta h^*}{RT} + \frac{(1-b)\Delta h^*}{RT_f}\right\},$$

where a_i, and b are constants and Δh^* denotes the activation enthalpy. Another expression for the relaxation time was obtained in Ref. [2]. In the work cited, the vitrification of the originally fluid state was described on the basis of a kinetic equation for a first-order chemical reaction (weak cooperative transition) or second-order reaction (strong cooperative transition). The inverse relaxation time has been defined as a sum of the probabilities of the fluid to solid state transition and vice versa:

$$\tau^{-1} = w_{fs} + w_{sf} = c_1 \exp(-u/kT)$$

or $\tau \approx \tau_0 \exp(u/kT)$. This also implies an activated behavior for the inherent properties of the vitreous fluid, e.g. for the viscosity [60]. The activation energy u has been predicted [60] to be dependent on the compressibility of the system, which induces a slight temperature-dependence.

In the membrane systems considered above, the phase behavior is determined by several interdependent processes which may be expressed in terms of characteristic times of different relaxation phenomena. Then, the existence of a time hierarchy can lead to the nontrivial kinetics of phase transformations. When the temperature of a supercooled system changes with time

$$q = \Delta T/\Delta t = \text{const},$$

isobaric cooling (or heating) is determined as a limit $\Delta T \to 0$ of the set of small instant temperature variations ΔT at the time interval Δt. In accordance with classification of Ref. [6], there exist three intervals

$\Delta t \gg \tau$ liquid state;

$\Delta t \sim \tau$ transition region;

$\Delta t \ll \tau$ glass state.

For elastic water-permeable membranes it is possible to mark three characteristic times of structural rearrangement at the cooperative liquid crystalline-to-gel phase transition. From experimental FRAP (fluorescence after photobleaching) data [61], τ_1 ranges at the time interval $\tau_f = 0.22$ s; $\tau_{fs} = 1.17$ s; $\tau_s = 6.97$ s. The second characteristic τ_2 is a viscous elastic membrane response to osmotic swelling–shrinkage which is dependent on water diffusion through the membrane and the geometrical size of the liposome (10^{-3} s to 1 s, $r \sim 0$, $1\mu \div 1\mu$, egg PC (phosphatidylcholine)) [50]. The last characteristic time τ_3 is the pore formation time. This τ_3 has been estimated in Ref. [50] to be from 10^{-3} to 10 s for $r \sim 0$, $1\mu \div 1\mu$, egg PC. From this it follows that the viscous elastic "tail" of the membrane response to rapid temperature perturbations can stretch and slow down the PT kinetics or shift the PT temperature. Besides, a prolonged period of large pore formation has suggested the idea of preventing membrane rupture by rapid cooling [56]. The idea was to achieve surface glass-like state during the defect preincubation (lag) period, before membrane rupture.

6. Concluding remarks: cryopreservation aspects

Any liquid on rapid cooling would solidify to a crystalline state or form a glass if crystallization did not intervene [6]. The relationship between the rate of cooling and the relaxation time of the system determine whether or not the liquid forms a glass [3]. Glass formation on fast cooling of a cell suspension has led to the idea of cryoprotection [62, 63] when cell injury as a result of ice crystallization is retarded.

It is known, that living cells can survive at optimum cooling and warming rates if they are protected by low toxity substances (cryoprotectors) added before freezing at the boiling point of liquid nitrogen [62–65]. However, even in the presence of cryoprotectors damage can still occur. When cooling is relatively slow, cells are killed by the high salt concentrations owing to intracellular water loss as a result of osmosis [63]. In contrast, when a cell suspension undergoes fast supercooling, enclosed water has insufficient time to flow out of the cells and they can be killed by intracellular ice crystallization [63]. They demonstrate good viability at intermediate cooling rates where the effects of damaging salts are minimal. This implies an optimum cooling–warming rate at which both temperature and osmotic stresses seem to be taken into account.

It is believed that cryoprotectors work against ice crystallization damages in two ways. Firstly, they reduce the temperature of equilibrium ice crystallization. Secondly, at high cryoprotector concentrations, rapid cell undercooling–rewarming causes many fewer injuries because the outside solutions remain wholly amorphous or the ice crystals are too small [64, 65].

Looking at the matter in another way, the cell damage caused by cooling before the crystallization stage (the so-called "thermal shock") is connected with the processes of phase separations of lipids and proteins in cell membranes above 0°C. During the lipid main phase transition (PT) the cooperative structural reconstructions from the initial liquid crystalline ("fluid") to gel ("solid") state take place.

The liquid crystalline state is mesomorphic. The main feature of such a phase is fluidity, i.e. it has rheological properties like liquid. It has been supposed [18] that such fluid membrane would solidify to a glass-like state on rapid supercooling. As a lipid membrane also has elastic properties, the tension arising can shift the "vitrification" temperature T_g towards the main PT region. This phenomenon, referred to as membrane "vitrification", is a two-dimensional analogy of the liquid–glass transition in commonly used vitreous media. Important theoretical consequences must be emphasized.

(1) There exists a critical cooling rate q at which the growth of new (gel) phase is halted or membrane "vitrification" would lead to the formation of very small domains instead of large. This q_c value depends on water diffusion through the membrane, membrane composition, its geometric size, and its elastic modulus.

(2) As a consequence of sharp slowing down of the membrane volume kinetics and corresponding surface area change at $q > q_c$, the formation of a large hydrophilic pore and following membrane rupture can be prevented during membrane "vitrification" where such a defect has no time achieve a critical radius. Thus, the idea of membrane "vitrification" sets a problem of protection at membrane level.

Further advancement of the theory may be also important from the standpoint of the optimum storage of lipid emulsions. Apart from the intriguing physics of closed lipid membranes (liposomes) they serve both as cells models and drug carriers. The functioning of such a liposome capsule is strongly dependent on its phase state [24]. Thus, theoretical prediction of the nonlinear membrane response at highly nonequilibrium conditions would be an effective means of controlling membrane phase behavior.

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References

[1] J.N. Gibbs and E.A. Di Marzio, J. Chem. Phys., 28 (1958) 373.
[2] M.V. Vol'kenstain and O.B. Ptitsyn, Sov. Phys. Tech. Phys., 1 (1957) 2138.
[3] D. Turnbull and M.H. Cohen, J. Chem. Phys., 34 (1961) 121.
[4] R. Davies and G. Jones, Adv. Phys., 2 (1953) 370.
[5] J. Šesták, Thermochim. Acta, 95 (1985) 459
J. Sěsták, in Z. Chvoj, J. Šesták and A. Třiska, (Eds.), Kinetic Phase Diagrams, Elsevier, Amsterdam, 1991.
[6] C.A. Angell and K.J. Rao, J. Chem. Phys., 57 (1972) 470.
C.A. Angell, Phys. Chem. Solids, 49 (1988) 863.
[7] J. Jackle, Rep. Prog. Phys., 49 (1986) 171.
[8] W. Gotze, in J.P. Hansen, D. Levesque and J. Zinn-Justin (Eds.), Liquids Freezing and Glass Transition, North-Holland, Amsterdam, 1991, p. 289.
[9] W. Gotze and L. Sjogren, Rep. Prog. Phys., 55 (1992) 241.
[10] L. Sjogren and W. Gotze, J. Non-Cryst. Solids, 7 (1994) 172–174.
[11] W. Gotze, Liquid–Glass Transition, Moskow, Nauka, 1992, p. 192.
[12] M. Elmroth, L. Borjesson and L.M. Torell, Phys. Rev. Lett., 68 (1992) 79.
D.L. Sidebottom, R. Bergman, L. Borjesson and L.M. Torell, Phys. Rev. Lett., 68 (1992) 3587.
[13] P.N. Pusey and W. Van Megen, Proc. 12th Gen. Conf. Cond. Mat. Div. of EPS, 6–12 April, Praha, 16A (1992) 13.
G. Szamel and H. Lowen, Phys. Rev. A: 44 (1991) 8215.
[14] R. Kubo, Statistical Mechanics, North-Holland, Amsterdam, 1965, 452.
[15] J. Keizer, Statistical Thermodynamics of Nonequilibrium Processes, Springer, New York (Berlin Heidelberg London Paris Tokyo), 1987, p. 608.
[16] M.V. Voinova, Colloids and Surfaces A: Physicochem. Eng. Aspects 95 (1995) 133.
[17] P.G. de Gennes, Phys. Lett. A., 47 (1947) 123.
[18] J. Nagle, Phys. Rev. A: 37 (1988) 3993.
[19] J.N. Israelachvili, D.J. Mitchell and B.W. Ninham, J. Chem. Soc. Faraday Trans. 2. 72 (16) (1976) 1525.
J.N. Israelachvili, S. Marcelja and R.G. Horn, Quart. Rev. Biophys., 13 (1980) 121.
P.A. Pincus and S.A. Safran, J. Chem. Phys., 86 (1987) 1644.
R.P. Rand, N.L. Fuller, S.M. Gruner and V.A. Parsegian, Biochemistry, 26 (1990) 76.
V.A. Parsegian, R.P. Rand, in J. Wilschut and D. Hoekstra, (Eds.), Membrane Fusion, Marcel Dekker, Inc., 1991, 65.
[20] M.V. Voinova and A.M. Kosevich, The Lars Onsager Symp., Coupled Transport Processes and Phase Transitions, Trondheim, Norway, June 2–4, 1994, Lerchendal Gard, Tapir, eds., Trondhiem, 1993, 124.
[21] O. Kedem and A. Katchalsky, Trans. Faraday Soc., 59 (1963) 1918.
[22] A. Kotyk and K. Janacek, Membrane Transport. An Interdisciplinary Approach. Plenum Press, New York, London, 1977, p. 341.

[23] D. Marsh, A. Watts and P.F. Knowles, Biochemistry, 15 (1976) 3570.
[24] M.B. Yatvin, J.N. Weinstein, W.H. Dennis and R. Blumental, Science, 202 (1978) 1290.
[25] H. Trauble, J. Membr. Biol., 4 (1971) 193.
[26] J.F. Nagle, Biochim. Biophys. Acta, 513 (1978) 236.
[27] M.C. Block, E.C.M. Van-der-Nout-Kor, L.L.M. Van Deenen and J. De Gier, Biochim. Biophys. Acta, 406 (1975) 187.
[28] D. Papahadjopoulos, K. Jacobson and T. Isak, Biochim. Biophys. Acta, 311 (1973) 330.
[29] J.E. Lovelock, Nature, 173 (4406) (1954) 659.
[30] P. Mazur, S.P. Leibo and E.H.Y. Chu, Exp. Cell. Res., 71 (1971) 345.
[31] H.T. Meryman, Ann. Rev. Biophys. Bioenerg., 3 (1974) 341.
[32] V.G. Ivkov and G.N. Berestovsky, Dynamical Structure of Lipid Bilayers, Nauka, Moskow, 1981, p. 224.
[33] M.V. Voinova, V.L. Galkin and A.M. Kosevich, Bioelectrochem. Bioenerg., 24 (1990) 143.
[34] E.A. Evans and R. Skalak, Mechanics and Thermodynamics of Biomembranes, CRC Press, Inc., Boca Raton, FL, 1980, p. 304.
[35] W. Helfrich, Phys. Lett. A., 50 (1974) 115.
[36] W. Helfrich, in J. Charvolin, J.F. Joanny and J. Zinn-Justin (Eds.), Liquids at Interfaces, Session 48, Les Houches, 1988, p. 212.
[37] V. Romero-Rochin, C. Varea and A. Robledo, Phys. Rev. A: 44 (1991) 8417.
[38] G. Cevc and D. Marsh, Phospholipid Bilayers. Physical Principles and Models, Wiley, New York, 1987, p. 548.
[39] V. Heinrich, S. Svetina and B. Zeks, Phys. Rev. E: 48 (1993) 3112.
[40] L.J. Lis, M. McAlister, N. Fuller, R.P. Rand and V.A. Parsegian, Biophys. J., 37 (1982) 667.
[41] A.G. Lee, Biochim. Biophys. Acta, 472 (1977) 237.
[42] T.Y. Tsong and M.I. Kanehisa, Biochemistry, 16 (1977) 2674.
[43] J.F. Nagle, Ann. Rev. Phys. Chem., 31 (1980) 157.
[44] S. Marcelja, Biochim. Biophys. Acta, 367 (1974) 165.
[45] F. Jahnig, Mol. Cryst. Liq. Cryst., 63 (1981) 157.
[46] V.S. Markin and M.M. Kozlov, Biophyzika (USSR) 28 (1983) 1031.
[47] M. Avrami, J. Chem. Phys., 7 (1939) 1103.
[48] P.G. De Gennes, The Physics of Liquid Crystals, Oxford, 1974, p. 357.
[49] C. Taupin, M. Dvolaitsky and C. Sauterey, Biochemistry, 14 (1975) 4771.
[50] M.M. Kozlov and V.S. Markin, Biol. Membr. (USSR) 1(1) (1984) 74.
[51] Ya.B. Zel'dovich, Zh. Eksp. Teor. Fiz, 12 (1942) 525.
[52] B.V. Deryaguin and Yu. V. Gutop, Kolloidn. Zh. 24 (1962) 431.
[53] A.G. Petrov, M.D. Mitov and A.I. Derzhanski, in L. Bata (Ed.), Advances in Liquid Crystal Research and Applications, Pergamon Press, Oxford – Academiai Kiado, Budapest, 1980, p. 695.
[54] I.G. Abidor, V.B. Arakelyan, V.F. Pastushenko, M.R. Tarasevich, L.V. Chernomordik and Yu.A. Chizmadzhev, Doklady Akad. Nauk, SSSR, 240 (1978) 733.
V.F. Pastushenko, Yu.A. Chizmadzhev and V.B. Arakelyan, Bioelectrochim. Bioenerg., 6 (1979) 53.
[55] M.V. Voinova, Kriobiologia (USSR), 4 (1985) 51.
[56] M.V. Voinova and A.M. Kosevich, J. Phys. (Paris), submitted for publication.
[57] A. Miller, W. Knoll and H. Mohwald, Phys. Rev. Lett., 56 (1986) 2633.
[58] C.T. Moynihan, A.J. Easteal, J. Wilder and J. Tucker, J. Phys. Chem., 78 (1974) 2673.
[59] C.T. Moynihan, A.J. Easteal and M.A. De Bolt, J. Am. Ceram. Soc., 59(1–2), (1976) 12.
[60] L. Sjogren, J. Phys. IY C1 (France), Suppl. J. Phys. II, 3 (1993) 117.
[61] W.L.C. Vaz, E.C.C. Melo and Th.E. Thompson, Biophys. J., 56 (1989) 869.
[62] G.J. Morris, in G.J. Morris and A. Clarke (Eds.), Effects of Low Temperatures on Biological Membranes, Academic Press, London, 1981, p. 262.
[63] J. Farrant, in M.J. Ashwood-Smith and J. Farrant (Eds.), Low Temperature Preservation in Medicine and Biology, Pitman Medical, 1980, p. 1.
[64] G.J. Morris and J. Farrant, Cryobiology, 9 (1972) 173.
[65] P. Boutron, J. Phys. Chem., 87 (1983) 4273.