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Cryogels on the basis of natural and synthetic polymers: preparation, properties and applications

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Abstract. This review deals with cryotropic gelation in polymeric systems, which takes place on moderate freezing, storing in a frozen state and subsequent thawing of solutions or colloidal dispersions containing monomeric or polymeric precursors. The polymer materials formed under these conditions are termed as cryogels. They are macroporous heterophase gels in which polycrystals of the frozen solvent act as porogens during gel formation. The current knowledge about the structure of moderately frozen solutions of low- and high-molecular-mass compounds is considered, the general regularities of the cryotropic gelation processes and their similarity to and differences from gelation at positive temperatures are discussed, the fields of application of cryotropic gelation techniques are described, and examples of using polymeric cryogel-based materials for solving applied problems are given. The bibliography includes 433 references.

I. Introduction

The applications of processes based on freezing are quite diverse. They include food technology,[1] ground freezing for the construction of underground utilities, the formation of cryobanks for storage of biological objects (blood, semen), ice preparation in sports centres, cryoconcentration of brines in chemical technology and fruit juices during processing of agricultural products, freeze-drying (lyophilisation) of drugs and many other. Branches of science such as low-temperature physics, cryobiology and cryomedicine, geocryology, glaciology, *etc*., are developing. Cryochemistry is in progress,[2] including those fields concerned with frost resistance of polymers, low-temperature polymerisation, cryoresistance of latexes, *etc*. This review is devoted to one line of research in polymer cryochemistry, namely, cryotropic gelation processes. Despite the fact that systematic studies along this line were started only in the 1970s, the number of patented processes and materials has already exceeded several hundred. However, no surveying papers have been published so far. All known reviews are concerned either with particular cases of gelation (for example, radiation-induced polymerisation in frozen solutions giving rise to cross-linked gel materials[3]) or with only one type of gel [in particular, the macroporous gels resulting from freezing – thawing of concentrated solutions of poly(vinyl alcohol) (PVA)[4–11]]. The main goal of this review is to summarise and describe systematically the information on the cryotropic gelation in general and on the specific features that distinguish it from the processes traditionally used to prepare chemical (covalently cross-linked) and physical (non-covalent) gels.

The explanation of the main notions and terms such as cryotropic gelation, cryostructuring and cryogel(s), *etc*., can be found in the literature.[6,9,10,12–14] In addition, the author proposes that the processes yielding gels of various natures would be termed by words with Greek or Latin roots constructed as follows: '... + tropic' [from τρωπωσ (tropos), which implies directed or induced[12]], *e.g*., chemotropic — induced by the formation of chemical bonds between polymer chains and resulting in a three-dimensional covalent network of the gel; ionotropic — induced by ion exchange (the formation of ionic bonds); chelatotropic — induced by chelation; thermotropic — induced by heating; psychrotropic — induced by cooling without freezing [from ψιχρια (psychria), which means chill[12]], by analogy with the well-established terms such as psychrometer, a psychrophilic microorganism, *etc*.; cryotropic — induced by freezing; solvotropic (or solvatotropic) — induced by a change in the solvent properties (quality).

The notions of positive and negative temperatures (referred normally to 0 °C) and the notions of moderately frozen systems also require more accurate definition. As applied to the objects discussed in this review, positive temperatures are those above the freezing point of any solvent, while negative temperatures are those below this point. Systems are considered to be moderately frozen if they retain a fraction of non-frozen solvent (usually, this refers to a temperature region lower than the freezing point of the neat solvent by not more than several tens of degrees).

II. What are cryotropic gelation and cryogels?

The structured polymeric physical bodies called gels (or lyogels, in colloid chemistry) can be characterised as immobilised solvent – polymer systems, in which macromolecules are connected in a three-dimensional network by non-fluctuating bonds that are rather stable in time. The nature of these bonds and the network morphology (single-phase and heterophase systems are the 1st-

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and 2nd-kind gels, respectively, according to Papkov's classification [15]) are determined by the chemical structure and the method of preparation of the gel. The solvent retained by the cross-linked polymer plays a very important role, as it prevents the system from collapsing into a compact mass and ensures diffusion of soluble substances from the surrounding liquid medium into the gel and back.[16–18] Gels, except for thixotropic ones, are normally able to undergo substantial reversible deformations in the absence of a flow.[19]

Two basic methods for the preparation of gels are known [15,20] to exist (Fig. 1): first, swelling of either a polymer (as a block, a film, a fibre or a powder) or a xerogel (cross-linked polymer without a solvent) in an appropriate low-molecular-weight liquid; second, gel formation in a liquid medium (solution or colloid dispersion of the corresponding gel-forming agents).

In the former case, a gel is formed due to limited swelling of a non-cross-linked polymer or swelling (exhausting swelling, if the equilibrium is reached) of a xerogel, which, in turn, can be prepared by a solvent-free synthesis or by mere drying of a lyogel.

The latter case represents the most widely used route to gel formation. In this case, the initial system can be a solution of monomers in which branching polymerisation or polycondensation is initiated; or a solution of high-molecular-weight compounds containing appropriate cross-linking agents (ionotropic and chelatotropic types of cross-linking are possible); or a solution of a polymer converted into a physical gel due to a change in the thermodynamic quality of the solvent (by which, one means its affinity towards the polymer) or to partial crystallisation of the polymer; or colloidal sol, which undergoes a phase transition to a gel-like state.

Starting from these precursors, one can prepare chemotropic (covalently cross-linked) gels, ionotropic and chelatotropic gels (in which the three-dimensional network junction knots are stabilised by slightly dissociating salt or coordination bonds, respectively), and also thermotropic and psychrotropic non-covalent (physical) gels. Ageing of colloidal sols usually gives gels of a sophisticated morphology (coagulation and condensation-crystallisation gels, according to Rebinder [21]).

It was shown [22] that all types of gelation that take place in a solvent can also be realised in a cryogenic version for the corresponding initial systems using appropriate conditions for freezing, storing in the frozen state, and thawing (see Fig. 1). Thus, cryotropic gelation is a specific type of gelation that takes place upon cryogenic treatment of the initial systems potentially capable of forming a gel. A necessary condition for the processes resulting in the formation of cryogels is crystallisation (freezing) of the main bulk of the low-molecular-weight liquid present in the initial system.

The polymer gel phase can be formed in one of the stages of cryogenic treatment: either directly during freezing of the initial system [this refers, for example, to colloid dispersions of gelatinised starch [23] or to aqueous solutions of polygalactomannan from the locust bean gum (LBG), which is a block polysaccharide giving rise to thermally reversible physical cryogels],[24–26] or during storage of samples in the frozen state (this mainly refers to the formation of chemically cross-linked cryogels [14,27–30]), or during thawing of the frozen specimens (this is typical of non-covalent cryotropic gelation of, for example, aqueous solutions of PVA [9,31–34]). This will be considered in more detail below in the discussion of the cryotropic gelation kinetics.

First, we shall consider the structure and properties of moderately frozen solutions of low- and high-molecular-weight compounds in order to formulate the current views on the medium in which the cryogel formation proceeds.

III. The structure of frozen solutions of low- and high-molecular-weight compounds. The notion of non-frozen liquid microphase (NFLMP)

The freezing and melting (thawing) of frozen solutions of various substances are fairly complex phenomena dependent on a large number of factors. Although studies in this field have long been carried out, new data providing more precise and more in-depth understanding of the mechanisms of these processes constantly appear in scientific literature. The progress of these studies is promoted to a large extent by the advent of experimental instrumentation and involvement of new methods and theoretical approaches. It is noteworthy that the traditional views according to which frozen solutions are polycrystalline solids at temperatures below the eutectics cannot explain, for example, the occurrence of chemical reactions with diverse mechanisms in these systems. These views do not allow interpretation of the spectroscopic data (ESR, NMR) concerning the maintenance of the high mobility of some of the solute and solvent molecules down to temperatures tens of degrees lower than the freezing point of the initial system.

The cryotropic solidification of neat crystallisable liquids and solutions of low-molecular-weight compounds (LMWC) in such liquids has been studied most extensively, mainly under conditions close to equilibrium.[35–37] However, freezing of these objects under conditions far from equilibrium brings about specific

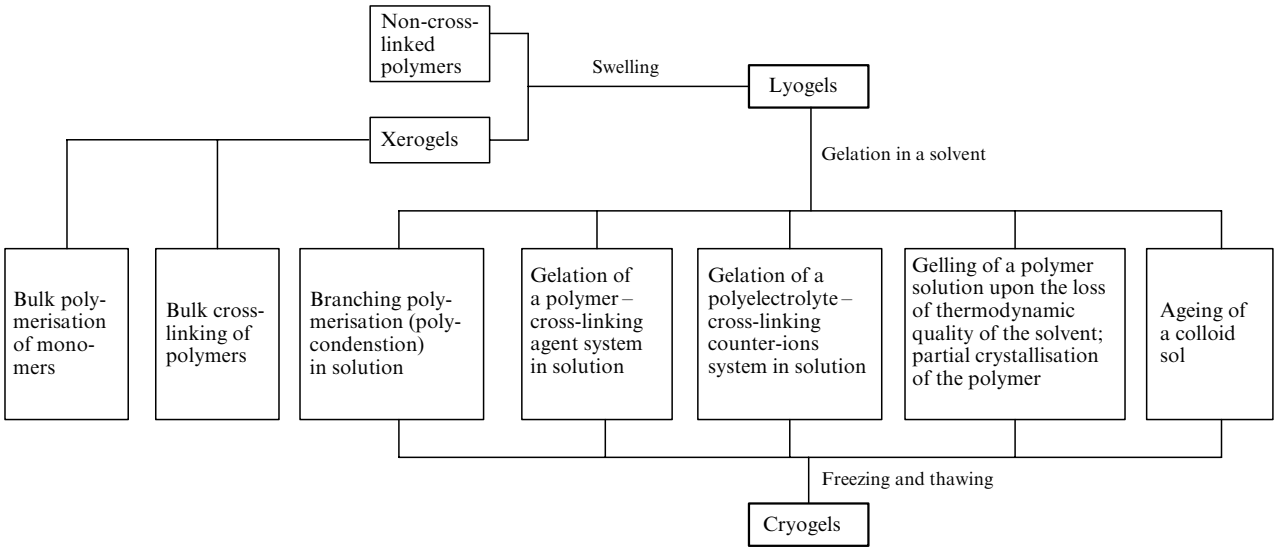


Figure 1. Generalised chart of the techniques for the formation of polymer gels.

effects, depending on the cooling rate, the specimen geometry, the vessel material, the duration of storage in the frozen state, and lots of other factors.[38–41] The processes taking place during the cryogenic treatment of solvent–polymer, solvent–polymer–monomeric additive, and solvent–swollen gel systems are even more complex. The course of freezing or thawing of such systems is strongly influenced by the polydispersity of the polymers (an exception is represented by narrow homogeneous fractions of molecular-weight standards or purified protein specimens). This gives rise to non-equivalence of the liquid–solid phase diagrams for the same solvent–polymer pair characterised by different molecular weights (MW) or molecular-weight distributions (MWD) of the polymeric component. Due to the polymer polydispersity, Smith and Pennings[42] proposed that the solvent–polymer systems subjected to freezing should be called pseudo-binary, to distinguish them from the solvent–LMWC binary systems. In addition, due to the slow relaxation processes in high-viscosity media, the attained phase states of the frozen specimens depend appreciably on the thermal pre-history during freezing. The capability for association in solutions at low temperatures, inherent in many polymers, results in the situation that the phase diagram of one system at the same negative temperature would depend in each particular case on the dynamics of cooling of the given specimen and the rate of solvent crystallisation.

In addition, high rates of liquid crystallisation in solutions and, especially, in swollen gels bring about the risk of mechanical destruction of polymer chains (so-called cryocracking) under the action of shear or wedging stresses in the zones of movement of the solvent crystallisation fronts (see, for example, Refs 43–46).

Below we mainly consider the phenomena that are significant for the cryotropic gelation processes. The anomalous depression of the freezing temperature of cross-linked gels [44,47–52] or the effect of the polymer MW and concentration on the trend of its solution to undergo either crystallisation or glass transition on cooling[53] are beyond the scope of this review, as well as some other aspects concerning freezing of polymer solutions and gels but not related directly to cryostructuring processes.

1. The liquid microphase in frozen multicomponent solutions

The pioneering studies demonstrating the possibility of chemical reactions in moderately frozen solutions (*i.e.*, in systems retaining a fraction of non-frozen solvent) were published in the 1960s.[54–60] Grant *et al.*[54] discovered a higher (compared to that at room temperature) degree of hydrolysis of the β-lactam ring in penicillin G and 6-aminopenicillanic acid in the presence of alkaline catalysts in frozen aqueous solutions of the reactants. The highest degree of conversion was observed at −18 °C. Study of the reaction products showed[55] that after thawing, the solution of 6-aminopenicillanic acid contained 7–8-unit oligomeric peptides. These were not formed in a similar solution kept at 20 °C for the same period. Thus, oligomerisation took place only on cryogenic treatment. Similar results have also been obtained in the synthesis of oligopeptides by polycondensation of *N*-carboxyanhydrides of several amino acids in frozen dioxane (T_m = 11.4 °C).[56] This process was most efficient in the crystallised reaction system at 1 °C rather than at positive temperatures.

Butler and Bruice [57,58] were, apparently, the first to suggest that the systems obtained by moderate freezing of multicomponent solutions of LMWC contain, after macrosolidification, apart from the solid phase, liquid regions between the solvent crystals in which the reactants are accumulated. Subsequently, this idea was further developed in Pincock's and Kiovsky's works (see, for example, reviews [59,60]). Having studied the kinetics of some usual (*i.e.*, not involving oligomer or polymer formation) reactions of LMWC in frozen solvents (both water and organic solvents), the researchers concluded that these reactions proceed in 'liquid holes, present among the crystalline solid, which contain high concentrations of the reactants'.[61]

This early stage of investigation resulted in the following conclusions:[60]

(1) moderate freezing of a solution of reactants in a crystallisable solvent does not terminate the reaction because substances are accumulated in the liquid regions; over a certain range of negative temperatures, this may even accelerate the reaction;

(2) if the total volume of the liquid inclusions remains unchanged during the reaction (the temperature does not change and the products and the reactants are in solution), the observed (apparent) rate (v_{obs}) of substance transformation can be described (with good agreement with experimental results) by the usual kinetic equation

$$v_{obs} = \frac{1}{V}\frac{dm}{dt}, \quad (1)$$

where V is the volume of the reaction phase, *i.e.*, the total volume of liquid inclusions; m is the number of moles of reactants in the reaction phase; and t is time. With v_{obs} determined by analytical methods, it is possible to estimate the V value;

(3) the dependence of the reaction rate on temperature for moderately frozen solutions does not obey the Arrhenius equation and passes through an extremum;

(4) reactions in moderately frozen media differ fundamentally from solid-phase reactions because the reactant molecules in the regions that remain liquid can execute forward motion, which is hardly possible in a solid (at both macroscopic and microscopic levels).

A significant contribution to the development of the concept of structural and phase inhomogeneity of multicomponent frozen solutions (in particular, at temperatures below eutectic ones) was made by Sergeev and Batyuk (see reviews [2,62–64]). They confirmed that frozen solutions actually contain regions, the mobility of molecules in which is comparable with that in liquids, and developed kinetic models suitable for quantitative description of reactions proceeding in such systems.

Ultimately, it was established quite definitely that moderately frozen solvent–LMWC systems are not entirely solid not only in the phase diagram region between the liquidus and solidus lines but also well below this region. In non-solidified regions which Sergeev and coworkers called the 'non-frozen liquid microphase' [65] (NFLMP), the molecules possess high mobility; this shows itself as narrow signals in high-resolution NMR spectra. If the reactants are readily soluble, they are accumulated (in the limiting case, completely) in the NFLMP. From the thermodynamic standpoint, the formation of the liquid microphase in multicomponent frozen systems stems from the fact that 'inclusion of dissolved substances in the crystal lattice of a solid solvent requires more energy than that spent for the increase in the chemical potentials upon an increase in the concentrations of components in the liquid microphase'.[62]

The existence of NFLMP in frozen solutions of monomeric substances at temperatures below the eutectic point of the corresponding system can be detected, in particular, by examining the NMR spectra of dissolved substances [66,67] or ESR spectra of spin probes.[68–70] For example, the triplet signal for 2,2,6,6-tetramethyl-4-hydroxypiperidin-1-oxyl in liquid benzene (T_m = 5.5 °C) remains such in a frozen sample down to ~ −50 °C and degenerates completely into a singlet only at liquid nitrogen temperatures, due to the fact that the solid microenvironment of the nitroxide probe suppresses its rotation.[62] It was found[71] that hydrolysis of *p*-nitrophenyl acetate in aqueous salt systems (solutions of Na_2HPO_4 or $Na_2B_4O_7$) with an eutectic composition prepared beforehand occurs at temperatures at least 10 ° lower than the eutectic point; the liquid-phase nature of this process was proved by special experiments.

After a thermodynamic equilibrium has been reached, frozen systems in which no reactions take place are characterised by the same NFLMP composition independent of the way of freezing of the initial solution.[65] However, during cooling of the initial system to a specified negative temperature, every instant of time is matched by a particular phase state, which depends on the conditions of cooling. The farther the cooling conditions from

equilibrium, the more pronounced the changes which occur in the frozen system during relaxation to the thermodynamic equilibrium. In solutions of polymers frozen under non-equilibrium conditions, relaxation proceeds much more slowly than that in LMWC systems. It is noteworthy that the liquid – solid diagrams constructed for such non-equilibrium conditions of freezing (this is almost always the case for chemical reactions carried out in moderately frozen solutions) are called non-equilibrium state diagrams rather than phase diagrams,[39] and the former can differ appreciably from the equilibrium diagrams. All these specific features of the phase state of systems subjected to freezing are especially important for those cryochemical processes whose rates are commensurable with the dynamics of crystallisation of the initial solution in which the amount (volume) and composition of the liquid microphase vary continuously during freezing until a phase equilibrium is reached.

Thorough experimental research of a number of cryochemical reactions in frozen multicomponent solutions and theoretical analysis of the results made it possible to derive kinetic equation (2), which differs from the above Eqn (1), referring to the simplest case, and allows the calculation of the reaction rates in these systems.[2, 62 – 64]

$$v_{obs} = k^0 e^{-E/RT} \left[\frac{T_0 - T}{\Delta \sum_i C_i} \right]^{\left(\sum_j n_j - 1\right)} \Pi_j C_j^{n_j}, \qquad (2)$$

where k^0 is the pre-exponential factor; E is the activation energy of the reaction determined for the liquid medium; R is the absolute gas constant; T_0 is the crystallisation point for the neat solvent; T is the temperature of the frozen sample; Δ is the cryoscopic coefficient of the solvent; C_i is the concentration of substances (i components) in the initial solution; C_j is the concentration of a jth reactant in the non-frozen region of the system; n_j is the reaction order with respect to the jth reactant.

The structure – kinetic model, which underlies this theory of chemical processes in frozen multicomponent solutions, interprets the non-monotonic pattern of the temperature dependence of the rates of reactions (whose overall order is more than one) in these systems as being due to the competition of the opposite trends, namely, the increase in the reaction rate caused by cryoconcentration and the decrease in the rate following the decrease in the temperature.

For the temperature corresponding to the maximum reaction rate T_{max}, the following relation was derived:[2, 62 – 64]

$$T_{max} = \left\{ \frac{E^2}{4R^2 \left(\sum_j n_j - 1\right)^2} + \frac{ET_0}{R\left(\sum_j n_j - 1\right)} \right\}^{0.5} - \frac{E}{2\left(\sum_j n_j - 1\right)}. \qquad (3)$$

It can be seen from Eqn (3) that T_{max} decreases with an increase in the overall reaction order. In the case where all the reaction components are concentrated in the NFLMP, and conditions for fast attainment of T_{max} have been found, selective acceleration of one of the several reactions proceeding in parallel becomes possible (and this has been confirmed by experiments [63, 64, 72, 73]).

The deviation of the physicochemical characteristics of the NFLMP (viscosity, density, dielectric constant, heat capacity, *etc.*) from those of non-frozen solutions caused by the change in the microenvironment has an influence on the reactivity of the solutes and their capacity for association. This, in turn, has an impact on the kinetics of cryochemical reactions in frozen multicomponent solutions. The data presented above lead to the conclusion that for a large number of moderately frozen solutions of individual substances and mixtures, NFLMP exists over a fairly broad range of negative temperatures. Both the initial components and the reaction products (if any reactions proceed) are accumulated in this microphase (provided that they are sufficiently soluble). These reactions are liquid-phase processes, despite the fact that the frozen specimen as a macroscopic body looks like a solid.

The information concerning such cryochemical processes and their mechanisms available from the literature mainly deals with frozen solutions of LMWC. The behaviour of polymer – solvent systems under these conditions is much more complex; the polydispersity of polymers (see above) is not the only reason for this difference. Another reason is the fact that polymer macromolecules not only execute translational and rotational motions as a single whole. In addition, they (first of all, flexible-chain polymers) possess a large set of intramolecular conformational degrees of freedom, in particular, segmental mobility. This accounts for the high sensitivity of these systems to freezing regimes.

2. Non-frozen solvent in polymer solutions and gels at temperatures below the freezing point of the system.

In the subsequent Sections of this review we shall demonstrate, using a number of particular examples, how moderate freezing, storage in the frozen state and subsequent thawing of a gel-forming polymer system result in the formation of a cryogel. Even the mere formation of the gel indicates that the reactants present in a frozen system (including polymers) do not lose mobility after crystallisation of the main bulk of the low-molecular-weight solvent. At least, they retain sufficient mobility for participation in intermolecular interactions giving rise to cross-links (either covalent or not) to form the three-dimensional cryogel network. It is clear that this type of motion can occur only in the solvent region that has not crystallised (or vitrified), *i.e.*, in the NFLMP.

It is noteworthy that most of the publications dealing with the properties of NFLMP in frozen polymeric systems concentrate on the non-frozen solvent rather than on macromolecular components because the physical methods used in the studies [most often, NMR and DSC (differential scanning calorimetry)] provide information mainly about the solvent. However, in some recent publications, the behaviour of polymers under these conditions has also been studied (see below). Since the interest in this field is related, first of all, to the development of cryobiology, cryomedicine and food technology, aqueous systems were studied most often,[1, 9, 22, 37, 39, 63, 64, 67, 70, 74 – 83] although Oikawa and Murakami [52, 84] considered organic solvent – polymer frozen systems. It was found that, on moderate freezing of polymer solutions or gels, after crystallisation of the main bulk of the solvent, its certain fraction (which is, moreover, much greater than that in solvent – LMWC systems) is not incorporated into the solid phase. Further cooling of samples may result in freezing out of an additional amount of the liquid component but, nevertheless, some remains non-frozen.

Physicochemical studies (NMR, ESR, DSC) of these frozen polymeric objects show the presence of non-frozen solvent at temperatures several tens of degrees lower than the freezing point of the system. For example, this is manifested in the NMR spectra as relatively narrow signals of the corresponding nuclei.[39, 77, 79, 81, 85 – 88] The ESR spectra of the spin probes introduced into the polymer solution before freezing are typical of the liquid microenvironment of the probe;[70, 89 – 92] calorimetry also shows incomplete crystallisation of the initial liquid.[39, 74, 76, 93 – 111] The fraction of the solvent which remains non-frozen can be rather high. For example, for aqueous polymeric systems (solutions of swollen slightly cross-linked networks), the amount of non-crystallised water reaches 0.4 – 1.0 g per g of the polymer, depending on the temperature, and the layer of non-frozen water firmly fixed by the polymer is $\sim$1 nm thick, which corresponds to two or three molecular hydration shells.[39] This value is an average because it was obtained by dividing the amount of non-frozen liquid into the polymer weight in the initial system. This did not

take into account the preferred solvation by water of hydrophilic sites of the polymer chains and the tendency of hydrophobic regions to expel water, which is the case for heteropolymers such as proteins or so-called protein-like synthetic hydrophilic-hydrophobic copolymers.[112–114] Therefore, the real sizes (volumes) of the areas which remain liquid after crystallisation of the main bulk of the solvent in water–polymer systems appear to be greater. This, in turn, creates conditions for the retention of mobility of chains or chain segments in the non-frozen region of the solvent. This is confirmed by high-resolution NMR spectra of frozen solutions of PVA in D_2O, which resembled the usual liquid-phase ^1H and ^{13}C NMR spectra of PVA, although the signals were markedly broadened. Thus, under these conditions, the polymer retains, at least, segmental mobility.[81,87,88] Analogous results were obtained for frozen aqueous solutions of polyacrylamide.[67]

The data indicating that some enzymes do not lose activity in moderately frozen solutions[2,115–125] also attest to the maintenance of segmental mobility of polymer chains under these conditions, because the transformation of a substrate into a product normally requires a conformational change in the enzyme macromolecule (especially, in the vicinity of the active site).[126,127]

The volume of polymer-containing NFLMP in a particular range of negative temperatures can be estimated using a special procedure proposed by Mikhalev *et al.*[70,89–92] for processing of the ESR spectra of spin probes (stable nitroxides) introduced into the system subjected to freezing. Hysteresis phenomena were observed,[70] that is, the NFLMP volumes measured during freezing and thawing were unequal.

For frozen aqueous solutions of polyhydroxy polymers (in particular, polysaccharides), data on the properties of water molecules of the first solvation layer occurring in direct contact with the polymer OH groups have been obtained. These H_2O molecules connected to the polymer through hydrogen bonds do not participate in the crystallisation–melting phase transitions; when a definite negative temperature (so-called T_g' point) is attained, they are vitrified together with the polymer. This fraction of liquid is referred to as non-freezable solvent.[93–99] Normally, each hydroxy group of the polymer chain retains approximately one water molecule.[108] In the case of polyelectrolytes, the ionogenic groups retain even larger amounts of non-freezable water due to Coulomb forces.[99] It should be emphasised that the non-freezable solvent is not identical to the non-frozen solvent (which failed to crystallise under particular conditions). The amount of the latter depends on the temperature and thermal pre-history of a particular specimen. For example, for the frozen D_2O–PVA system, the non-frozen solvent is responsible for a relatively narrow signal in the NMR spectrum down to a temperature 55 ° lower than the freezing point of the system. Only on further cooling, is it possible to record a broad signal from the non-freezable solvation water.[81] In the general case, the non-freezable solvent is a constituent part of the non-frozen solvent.[102]

As noted above, the amount of solvating non-freezable solvent depends on the conformation of macromolecules, for example, on the density of packing of the polypeptide chain in the protein globule. Thus it was found by NMR spectroscopy[85] that in a solution of serum albumin frozen at −35 °C, the amount of non-freezable water is 0.30 g per g of the protein. If the same macromolecules have been treated with urea, the packing of the polypeptide chains becomes loose and the quantity of non-freezable water increases to 0.44 g per g of the protein.

Thus, the higher the degree of solvation of the polymer in the initial solution, the greater the fraction of non-freezable solvent in the crystallised sample at negative temperatures, because in this case, more groups with high solvent affinity in the polymer become accessible for the solvent molecules.

Indeed, determination [by DTA (differential thermomechanical analysis), DSC and dilatometry] of the dependence of the non-frozen portion on the initial concentration of poly(*N*-vinylpyrrolidone) in aqueous solutions frozen at various temperatures showed that the overall weight of the liquid that has not crystallised increases with an increase in the polymer content but the proportion of this liquid decreases to reach some limiting value, which corresponds to the amount of non-freezable solvent (Table 1).

Table 1. Dependence of the weight of water non-frozen at −45 °C on the concentration of poly(*N*-vinylpyrrolidone) in the initial solution.[128]

Concentration (%)	Weight of non-frozen water (%)	Relative fraction of non-frozen water /g of H_2O per g of the polymer
5.0	10.9	2.18
15.0	21.0	1.19
30.9	36.6	0.80
39.2	48.9	0.77
46.4	53.6	0.62

In the case of freezing of swollen cross-linked polymers, the behaviour of the NFLMP has a number of specific features. For example, in cross-linked polyacrylamide hydrogels (PAAG), the amount of non-frozen water obeys an almost linear dependence on the temperature of the crystallised specimen in the −8 to −60 °C range.[77,129] In the subzero temperature range (0 to −8 °C), differences in the mobility of molecules in the liquid part of the solvent depending on whether the temperature of measurements was approached during cooling or heating of the specimen were detected by relaxation NMR spectroscopy.[129] In the case of system cooling, the spin–lattice relaxation time (T_1) measured at −6 °C was 4 times as long as T_1 found for the system obtained by heating of an identical PAAG specimen from a colder state, *i.e.*, the plot for the temperature dependence of T_1 has a hysteresis loop.[129] Nearly the same picture is observed at subzero temperatures for frozen gelatin or α_s-casein hydrogels.[79] One reason for these hysteresis phenomena may be the fact that during heating, the diffusion of water molecules from the crystalline to liquid phase is hampered due to the presence of the polymer network.[77] This demonstrates once again the important role of conditions (cooling or heating) used to prepare frozen specimens for phase equilibration.

These effects are inherent in not only aqueous systems. A certain mobility of polymer chains in moderately frozen lyogels can also be detected in cross-linked polymers swollen in organic solvents. For example, measurement of the spin–spin relaxation times (T_2) of protons in a cross-linked rubber swollen in deuterobenzene (T_m = 6.8 °C) shows that down to ∼−33 °C, chain segments still execute thermal motion in the medium of the crystallised solvent.[130] It has been shown[52] using NMR spectra of benzene-d_6 (solid-echo technique) that the mobility of the solvent molecules is still retained at temperatures lower by 15–18 °C.

It should be emphasised that various NMR techniques are most useful for investigations of frozen solvent–polymer systems.

To conclude this Section, we would like to summarise the important features of cryotropic gelation.

1. Crystallisation of a monomeric liquid during freezing of polymer solutions is accompanied by a minor temperature depression, which differs only slightly from the crystallisation point of the pure solvent.

2. In moderately frozen multicomponent solutions, some of the low-molecular-weight liquid remains non-crystallised at temperatures below the eutectic point. The non-frozen solvent molecules retain rather high mobility; these microregions (NFLMP) of the macroscopically frozen solutions accumulate the solutes, which also possess certain molecular (in the case of LMWC) or segmental (in the case of polymers) mobility.

3. The volume of the NFLMP depends on the system temperature, concentration of soluble (or swollen) components and, if

the phase equilibrium has not yet been established, on the instant of the measurement.

4. Due to cryoconcentration effects and some other factors (for example, the increase in the dielectric constant of the medium on cooling), acceleration of chemical reactions can be observed in a definite range of negative (for the used solvent) temperatures.

IV. Effects inherent in cryogel formation processes

As noted in the previous Section, moderate freezing of solvent – LMWC and solvent – polymer systems gives rise to phase inhomogeneous systems which include a non-frozen liquid microphase. If the initial specimen contains gel-forming agents, the polymer framework of the corresponding cryogel is formed exactly in these non-frozen microregions of the frozen material. This is sketched in Fig. 2.[6]

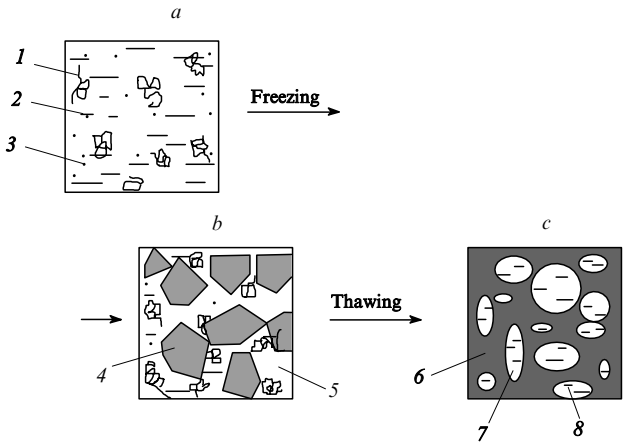


Figure 2. Basic diagram of cryotropic gelation.[6, 22]
(*a*) initial sample, (*b*) sample after freezing, (*c*) cryogel; (*1*) polymer precursors, (*2*) solvent, (*3*) low-molecular-weight compounds or monomer precursors, (*4*) polycrystals of the frozen solvent, (*5*) NFLMP, (*6*) polymer network of the gel phase of the heterophase cryogel, (*7*) macropores, (*8*) solvent.

The volume of NFLMP depends on numerous factors, in particular, on the nature of the solvent and the initial concentration and MW of the solutes (for polymers, also on the chain conformation), the system temperature, the regime of specimen freezing, the presence of soluble or insoluble admixtures, *etc*. In Fig. 2, all components are shown for clarity on an arbitrary (large) scale. It has been found [67, 70, 80 – 83] for several gel-forming systems that the NFLMP volume (Fig. 2 *b*) varies from fractions of a percent to several percent of the solvent volume in the initial specimen (Fig. 2 *a*). Thawing of the specimen gives rise to a macroporous cryogel (Fig. 2 *c*) containing large pores with variable size and geometry. The polycrystals of the frozen solvent (in some cases, crystal solvates or cryoprecipitate particles if these arise during cooling of the initial specimens) act as pore-forming agents (porogens) in this case. On melting or dissolution of these porogens after thawing, cavities filled with the melted solvent remain in the cryogel bulk. Due to the surface tension forces of the gel phase, the surface of the macropore walls is bent; the faceted shape of the walls, typical of the crystalline porogen particles, is replaced by a round shape. In addition, the wall material posseses its own (gel) porosity. Since this gel is formed at high concentrations of precursors, the resulting polymer phase is a dense network with a microporous structure.

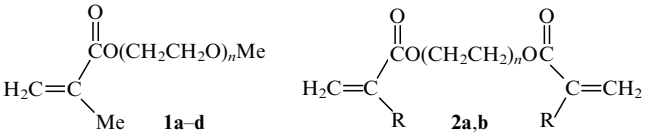
A typical feature of various polymeric cryogels is heterophase and heteroporous (often, supermacroporous, spongy or cellular) morphology. The macropores in the cryogel thus formed are usually interconnected because during freezing, each crystal of the solvent grows, as a rule, from the vessel periphery towards the centre (if no directional freezing is involved) until it comes into contact with the face of another crystal.

An increase in the precursor concentrations in the NFLMP (with respect to that in the initial system), *i.e.*, cryoconcentration is one of the main reasons for some specific effects typical of cryogel formation. A number of examples classified according to the types of gel-forming systems (see Fig. 1) are presented below.

1. Cryogels formed by polymerisation or polycondensation

The preparation of gel structures by polymerisation or polycondensation of monomer precursors is one of the most extensively used pathways to solvated polymer networks.[15 – 18, 131 – 134] Apparently, the first description of gels synthesised by polymerisation of unsaturated monomers in frozen media, *i.e.*, polymerisation cryogels, was described in publications [3, 135 – 138] devoted to the γ-radiation-induced gelation of frozen aqueous solutions of 2-hydroxyethyl methacrylate (2-HOEMA). It was found that at −24 °C and at an initial monomer concentration of 10% – 40%, turbid cellular (spongy) cryogels containing interconnected micrometer-size macropores are produced. When the monomer concentration is increased to 50% – 80%, a foam-plastic-like material with isolated macropores is formed, because the frozen system represents a vitrified matrix of the monomer with small ice crystals dispersed in it. When the initial content of water is low (< 10%), the process yields a transparent polymeric monolith because cooling of these specimens results in vitrification instead of ice crystallisation,[137] while polymerisation, evidently, occurs as a solid-phase process.[139] For the irradiation doses used (~1 Mrad) post-polymerisation does not occur in any of the systems, *i.e.*, the polymer is formed only during irradiation. The fact that a monomer with one double bond is converted into a cross-linked gel points to a branching mechanism of polymerisation, *i.e.*, chain transfer to a side functional group of the monomer takes place.

In addition to materials based on 2-HOEMA,[3, 4, 138, 140 – 165] a similar procedure is used to prepare macroporous cryogels based on oligo(ethylene glycol) methyl ether methacrylates **1a** – **d** [166] and oligo(ethylene glycol) diacrylates **2a** and dimethacrylates **2b**.[167 – 169]



n = 9 (**a**), 14 (**b**), 23 (**c**), 50 (**d**). R = H (**a**), Me (**b**); n = 2 – 14.

When the initial concentrations of the structurally related nine-unit mono- [166] and diacrylates [168] are identical, a lower swelling capacity is observed for cryogels based on the oligomer containing two double bonds, pointing to a higher degree of polymer cross-linking. The partial replacement of moderately hydrophilic 2-HOEMA in the initial composition by highly hydrophilic *N*-vinylpyrrolidone sharply increases the degree of swelling of the resulting cryogels in water and, as noted in a publication,[170] makes the polymer sponge 'softer'.

An interesting way of controlling the microporosity of the polymer phase in heterogeneous cryogels of this type has been reported.[171] Oligo(ethylene glycol) with an MW of 1000 Da is introduced as a 'molecular' porogen into the initial solution of a mixture of acrylic acid and methyl methacrylate. After radiation-induced cryopolymerisation of the unsaturated monomers, this additive is washed out of the gel phase by a large excess of water. Apart from the usual micropores present in the polymer network, this gel contains additional (a sort of calibrated) pores.

In order to synthesise cryogels with reactive aldehyde groups, acrolein is added to a solution of 2-HOEMA [or poly(oxyethylene dimethacrylates)]. Irradiation of the frozen solution of the comonomers affords a functionalised macroporous carrier suitable for

covalent coupling of specific antibodies for the preparation of immunosorbents.[172]

Thus, by varying the concentration and the nature of monomer precursors, one can change, over a wide range, the physical and chemical properties of the gels formed in the radiation-induced cryopolymerisation and used as carriers of immobilised enzymes,[140, 142, 149–153, 158, 160–164, 171] antibodies,[143] non-proteineous biopolymers[165] and microbial cells[141, 144–148, 156, 157, 159, 166] (see Section V).

Systematic studies of cryotropic gelation induced by polymerisation in frozen solutions induced by chemical initiators have been mainly carried out using polyacrylamide cryogels (cryoPAAG).[22, 29, 30, 173–177] The radical copolymerisation of acrylamide (AAM) with *N*,*N*′-methylenebis(acrylamide) (MBAAM) in water was initiated by the redox system used traditionally for this type of comonomer, namely, ammonium persulfate (APS) – *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), capable of generating radical ions, which initiate polymerisation even at low temperatures.[178]

The critical concentration for gelation (CCG) at 20 °C for a mixture of the AAM and MBAAM monomers in the synthesis of polyacrylamide gels (PAAG) amounts to ~2% (depending on the monomer ratio).[178] However, if the reaction mixture is frozen immediately after addition of the initiator (for example, kept at −10 °C for several hours) and thawed, it is possible to obtain cryoPAAG at a twice lower initial concentration of the same comonomers (Table 2).[22, 30] Thus, polymerisation cryogels can also be formed with chemical initiation; in this case, the CCG decreases. In reality, this decrease is only apparent, the main reason being the accumulation of reactants in the polymerisation region, *i.e.*, in the NFLMP. It is significant that this seeming decrease in the CCG is observed for any type of cryotropic gelation.

Table 2. Formation of polyacrylamide gels from low-concentration aqueous solutions of an AAM – MBAAM mixture at 20 and −10 °C in the presence of an initiator.[22, 173]

Total concentration of AAM and MBAAM /g dl^{-1}	The ratio [AAM] : [MBAAM] /mol mol^{-1}	Formation of PAAG or cryoPAAG	
		20 °C	−10 °C
1.0	50 : 1	−	+
1.0	40 : 1	−	+
1.0	30 : 1	−	+
1.0	20 : 1	−	+
1.0	10 : 1	−	+
2.0	50 : 1	−	+
2.0	40 : 1	−	+
2.0	30 : 1	−	+
2.0	20 : 1	+/−	+
2.0	10 : 1	+	+

Notes. Initiator: APS, 0.025 g dl^{-1} and TMEDA, 0.05 g dl^{-1}. The following designations are used: − means that the system remains liquid; +/− means that a very weak gel is formed; + means the formation of a gel or a cryogel.

Polyacrylamide cryogels have a spongy morphology, which is mainly dictated by the temperature regime of cryotropic gelation. Figure 3 presents the photomicrographs which show the structure of cryogels synthesised at −10 and −20 °C from identical solutions of comonomers frozen by different procedures: by placing the reactant solution into a cryostat with a specified temperature (freezing from above) (Fig. 3 *a*, *b*); and by initial fast freezing of the system in liquid nitrogen to −196 °C followed by transferring the specimens into a cryostat with a specified moderate negative temperature (Fig. 3 *c*, *d*). The latter procedure, which minimises the influence of the duration of cooling and freezing stages on the kinetics of cryochemical reactions, is sometimes called low-temperature quenching.

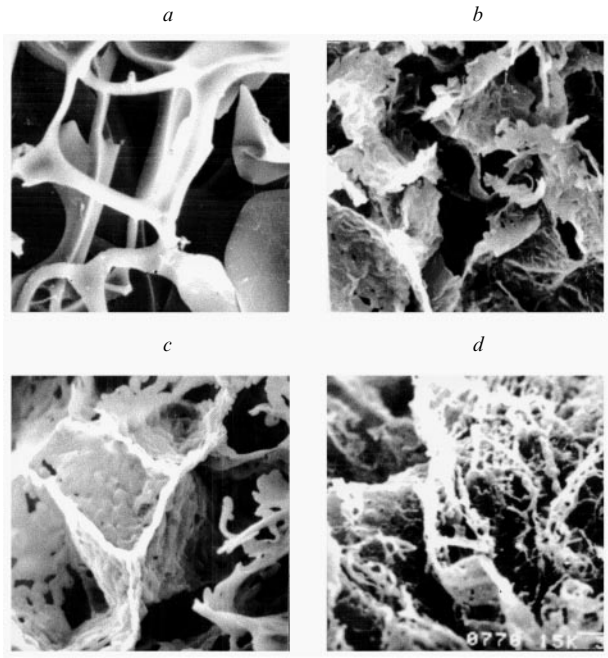


Figure 3. Electronic photomicrographs (scanning electron microscopy) of cryoPAAG specimens formed from aqueous solutions with a total comonomer concentration of 3% and [AAM] : [MBAAM] = 30 : 1 at different conditions of cryogenic treatment.[22, 176]
(*a*) Freezing from above, −10 °C; (*b*) freezing from above, −20 °C; (*c*) low-temperature quenching, −10 °C; (*d*) low-temperature quenching, −20 °C. Scale: 20 μm to 1 cm.

When cryogels are prepared by freezing from above, lowering of the synthesis temperature from −10 to −20 °C leads to a greater number of porogen particles (small crystals of ice) having, however, a smaller size; this has a noticeable influence on macropore diameter in the cryoPAAG (Fig. 3 *a*, *b*). In addition, the lower the temperature, the greater the amount of solvent that freezes out, *i.e.*, the NFLMP volume decreases, while the concentration of soluble substances in this phase grows. The walls of the macropores thus formed are thinner; however, according to measurements of the swelling capacity of the network material, they are built of a more concentrated gel.[22, 175] When the low-temperature quenching procedure is employed, the cryoPAAG morphology changes (Fig. 3 *c*).[22, 176] The cryogel architecture formed at −10 °C combines the porosity typical of the sample obtained by freezing from above at the same temperature and the structure of macropore walls inherent in the material prepared at −20 °C also by freezing from above. This means that, as the reacting system is warmed from −196 to −10 °C, the main structural elements of the gel phase have time to be formed during those several minutes when the system is at about −20 °C. Simultaneously, this indicates that polymerisation is rather fast under these conditions.

The porous structure of various cryogels can be characterised as an intricate three-dimensional maze. This is observed most often for aqueous systems due to the branched shape of ice polycrystals, which give rise to macropores of complex configurations in the specimen bulk. To prepare cryogels with a rather regular arrangement of macropores, it is necessary to use directional cooling and a solvent that forms oblong crystals on freezing. Thus formamide (T_m = 2.8 °C) crystallises as long thin needles. When cryoPAAG is prepared in this solvent and one end of the tube with the reaction solution is cooled, directional growth of formamide crystals takes place. The resulting cryogel contains long capillary channels oriented along the temperature gra-

dient.[22, 174] Thus, the macroporosity of the polymer cryogels can be 'governed' to a certain extent by selecting the solvent and crystallisation regime.

The osmotic characteristics (swelling behaviour in various media) of spongy cryogels similar to cryoPAAG are mainly determined by the initial concentrations of precursors, the nature of the solvent used, and conditions of cryogenic treatment. The total volume of the liquid absorbed by a cryogel during swelling consists of two components, namely, the solvating solvent firmly kept by the polymer network and the capillary solvent which fills the macropore space and can be removed rather easily even by slight compression of the cryogel. It can be seen from the photomicrographs shown in Fig. 3 that the latter component is markedly greater than the former one as the internal volume of macropores accounts for the greater part of the cryogel volume. For example, for the cryoPAAG specimen formed at -10 °C from a 3% solution of comonomers (in 40 : 1 molar ratio), the overall swelling capacity in water is ~ 80 ml per g of dry polymer, while the degree of swelling of the macropore wall material is only ~ 4 ml g^{-1} (see Ref. 173). The last-mentioned value corresponds to the swelling in water of usual PAAG prepared at positive temperatures from 10% – 20% solutions of the same precursors. This indicates that the polymer network of the cryogel is formed in the NFLMP, *i.e.*, in the medium highly concentrated as compared to the initial solution.

In this connection, of interest is one more specific effect inherent in the cryotropic gelation dynamics. Figure 4 shows[22, 176] the variation of the yield of the gel fraction during the PAAG and cryoPAAG formation at temperatures of 25 and 15 °C and at -15 and -25 °C. Cryopolymerisation was carried out using the freezing from above (Fig. 4 *a*) and low-temperature quenching procedures (Fig. 4 *b*). A mere decrease in the temperature of the process in the liquid solvent from 25 to 15 °C decreases the rate of PAAG formation and the yield of the gel fraction. Meanwhile, polymerisation in a moderately frozen system, in particular at -15 °C, is much faster than, for example, at 25 °C, and the final yield of cryogels is much higher. In other words, at definite negative temperatures, gelation is accelerated compared to the gelation of analogous specimens in a non-frozen medium. This phenomenon is typical of the formation of cryogels.

It is also noteworthy that the curves for the corresponding positive temperatures in Figs 4 *a*, *b* follow nearly the same pattern. This implies that the low-temperature quenching itself has no influence on the given reacting system; after fast freezing in liquid nitrogen and the subsequent fast warming of the reaction mixture, the usual gelation kinetics does not change (*cf.*, for example, the curves corresponding to 25 °C in Figs 4 *a* and 4 *b*).

The temperature dependences of the gelation rate in frozen media pass through extrema. The time it takes to reach the gel point (for given systems, this implies an approximately 10% yield of the gel fraction) actually reflects the process rate in the initial stage (Fig. 5). A decrease in the temperature in the positive region entails an increase in the gelation time, whereas in a certain range of negative temperatures in the frozen medium, the time required to reach the gel point sharply diminishes, *i.e.*, the rate of the formation of cryoPAAG increases. In the case of freezing from above, this temperature dependence has a clear-cut extremum, the initial gelation rate being a maximum in a fairly narrow range of negative temperatures. A somewhat different pattern is observed when low-temperature quenching is used, namely, the extremum is less pronounced and a high initial rate of the process is observed over a wider temperature range. This attests to non-equivalence of the conditions of copolymerisation of AAM with MBAAM in specimens brought to the reaction temperature along different routes. In other words, the process of formation of cryoPAAG depends on the method of freezing, although special experiments with a thermocouple embedded in the frozen specimen show[22, 176] that during the fast warming-up from -196 °C, the system temperature reaches a specified value before the gel point is attained. The non-equivalence of NFLMP characteristics in these reacting systems for the use of these freezing procedures is also indicated by NMR spectroscopy data,[67] which reveal the differences between the amounts of the solvent remaining in the liquid state in the specimens frozen by different procedures. Thus, in the case of relatively fast cryotropic formation of chemically cross-linked polymer networks, the regime of freezing of a reacting system is an important factor which influences, even at the stage of crystallisation, the properties and the formation dynamics of cryogels.

Nuclear magnetic resonance spectroscopy is one of the few physical methods that allow direct detection of chemical reactions

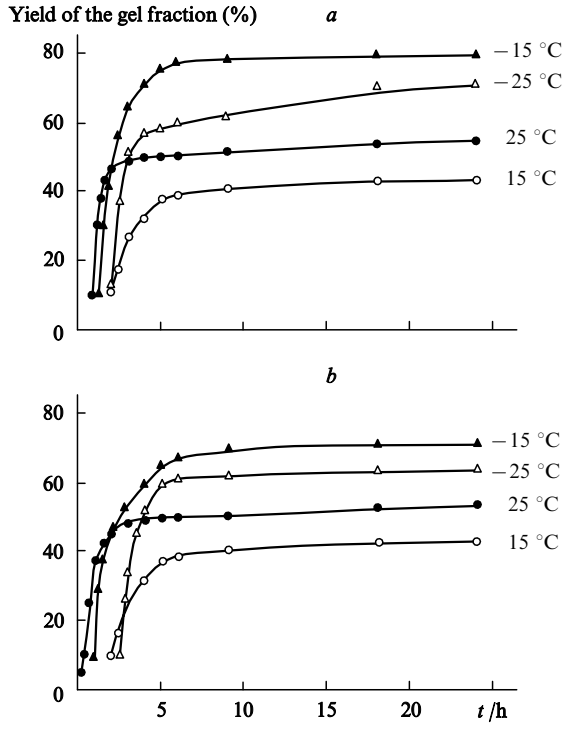


Figure 4. Dynamics of formation of PAAG and cryoPAAG (the temperature of synthesis is indicated near the corresponding curve) during freezing of the reacting system from above (*a*) and using the low-temperature quenching technique (*b*).[22, 176]
The initial total comonomer concentration is 3 mass %, [AAM] : [MBAAM] = 30 : 1.

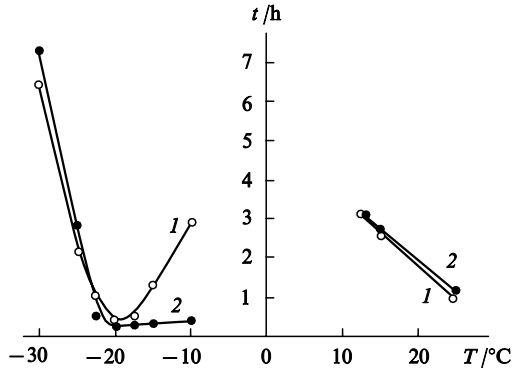


Figure 5. Time required for the formation of 10% of gel fraction in the reacting system *vs.* temperature of PAAG and cryoPAAG synthesis for freezing of the system from above (*1*) and for the low-temperature quenching version (*2*).[22, 176]
The initial total comonomer concentration is 3 mass %, [AAM] : [MBAAM] = 30 : 1.

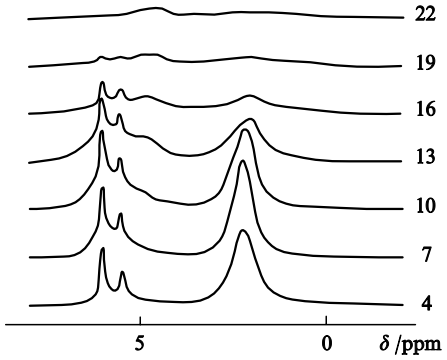


Figure 6. Evolution of the ^{1}H NMR spectra of a solution of a mixture of AAM, MBAAM, TMEDA, and APS in D_2O frozen using low-temperature quenching at -11.2 °C.[22,67]
The initial total comonomer concentration is 3 mass %, [AAM]:[MBAAM] = 30:1. The digits near the curves represent the period of time (min) from the instant the system reaches a specified negative temperature to the instant of recording the spectrum.

in moderately frozen systems without thawing the sample. For instance, the evolution of the ^{1}H NMR spectra recorded at definite intervals (Fig. 6) for a solution of reactants (AAM–MBAAM–TMEDA–APS) frozen in D_2O proves unambiguously that in this case, cryopolymerisation in the frozen medium takes place but neither pre- nor post-polymerisation during freezing or thawing, respectively. For an initial period, the spectra of the frozen solution of the reactants remain identical to the spectra of a similar specimen that does not contain the initiator. Subsequently (starting from about the tenth minute of the process), the intensity of the narrow signals due to the monomer protons [6.15 and 6.03 ppm (CH_2), 5.60 ppm (CH)] starts to decrease, and a broad signal [$\sim$5 ppm (CH_2)] for the protons of the polymer chains appears.[67] In addition, NMR spectroscopy shows that the reactant ratio in the NFLMP differs from this ratio in the initial solution due to the partial crystallisation of AAM and MBAAM at temperatures below the eutectic point; therefore, the reaction medium is enriched in the initiator (the spectra contain an intense common signal for the protons of the CH_3 and CH_2 groups of TMEDA with a maximum at $\sim$2 ppm). As polymerisation advances, the crystallised monomers are again dissolved in the liquid regions of the macrofrozen system and participate in the reaction, resulting ultimately in a high yield of the gel fraction. The measurement of spectroscopic characteristics of the non-frozen fraction of the solvent shows that an initial stage of polymerisation develops under conditions of increasing volume of the liquid microphase, and only when a certain degree of conversion of the monomers into polymeric products has been attained, does the solvent content reach a constant value.[67] Thus, depending on the cryopolymerisation temperature and freezing conditions, the composition and the volume of the reaction solution in the microreactor (non-frozen liquid microphase) can vary, which influences the gelation dynamics.

Since the variation of the temperature regimes of cryopolymerisation allows the macroporous morphology of gel materials to be controlled over certain limits, this approach has been used to prepare cryogels based on so-called stimuli-responsive [79] or sensitive [18] polymers. In recent years, gel materials prepared from these polymers have attracted increasing attention both for fundamental research and for applied purposes. In particular, hydrogels that collapse reversibly upon a change in the environment parameters (temperature, pH, ionic strength, thermodynamic quality of the solvent, applied electric field, and so on) are considered to be promising materials for biomedical and biotechnological purposes.[17,18,179–182] For example, in temperature-sensitive hydrogels in which the chains of the corresponding linear polymer undergo a reversible conformational change on passing through the upper or lower critical solution temperature (UCST or LCST), substantial thermally induced reversible changes in the degree of swelling take place. Hydrogels based on cross-linked poly(*N*-isopropylacrylamide), poly(*N*,*N*-diethylacrylamide) (PDEAAM), poly(*N*-vinylcaprolactam) and so on are examples of thermally sensitive polymer systems.[181–187]

Important characteristics of these materials which determine their performance characteristics include the temperature range in which the network collapses and the rate of system response to external treatment. Normally, the larger the specimen under study, the lower this rate, because the kinetics of collapse (or swelling) is controlled by heat transfer within the bulk of the gel and by the solvent diffusion and, hence, depends on the geometric dimensions of the specimen. However, if a macroporous structure is imparted to the material, the intrinsic response time of the macroporous specimen to a change in the temperature would decrease with respect to that observed for a fine-pored material, despite the fact that the weight and heat transfer rates in the gel phase remain the same. In this case, mass and heat transfer processes occur within the thin walls of macropores, *i.e.*, at short distances; as a consequence, the intrinsic time of collapse for this specimen is shorter than that for the microporous gel having the same total volume.

One approach to the synthesis of stimuli-responsive gels with enlarged pores is synthesis at temperatures above the collapse point of the cross-linked polymer (*i.e.*, above the LCST for the corresponding non-cross-linked polymer).[188–190] In this case, phase segregation results in the formation of a coarser-pored gel (with pores of up to several hundred of Ångsröm) compared to that prepared from the same solution of monomer precursors but in the homogeneous medium at temperatures below the LCST (with pores of several tens of Å). The use of auxiliary porogens, for example foaming agents,[191] at the stage of gel formation provides the possibility of producing an even coarser-pored material. In this case, some pores are closed but the rate of collapse or swelling of this gas-filled macroporous stimuli-responsive gel in reply to a change in the external conditions is much higher than that observed for homophase gel materials of the same chemical nature because the gel layer around each gas bubble is very thin.

As noted above, cryotropic gelation allows one to form gel systems with open interconnected capillary-sized macropores. Thus redox-initiated copolymerisation of *N*,*N*-diethylacrylamide or *N*-isopropylacrylamide with MBAAM in a frozen aqueous medium affords the corresponding macroporous (with pore size of several tens of micrometers) spongy temperature-sensitive cryogels.[192,193] Gel materials of this type, in particular PDEAAM cryogels, collapse on heating much faster (Fig. 7 *a*, curve *2*) than usual PDEAAM gels formed in the liquid medium (Fig. 7 *a*, curve *1*).[192] During the decrease in the swelling capacity, the main bulk of water retained by the cryogel is separated in the first 10–15 s. This includes almost all the capillary solvent and some fraction of the solvating liquid. The concentration of PDEAAM in the polymer phase of the cryogel (Fig. 7 *b*) reaches very high values (50%–70%), whereas for a collapsed usual gel, these values are lower (17%–42%). In other words, during the thermally induced decrease in the swelling capacity, a cryogel displaces more liquid from the polymer phase than the usual gel. At temperatures above the LCST and in the shrunk state, PDEAAM cryogels retain not more than 3.5–5 water molecules per monomer unit, while the polymer network of a usual gel with the same composition retains up to 10–35 water molecules per unit. A unit of PDEAAM has three lone electron pairs (two at the carbonyl oxygen atom and one at the amide nitrogen atom) able to form hydrogen bonds with three water molecules. Hence, it can be concluded that the degree of hydration of PDEAAM cryogels after the collapse is close to the lowest possible value. Thus, the use of cryotropic gelation techniques allows one to create a new type of gel material combining the properties of a network of stimuli-responsive gels and a supermacroporous structure typical of cryogels.

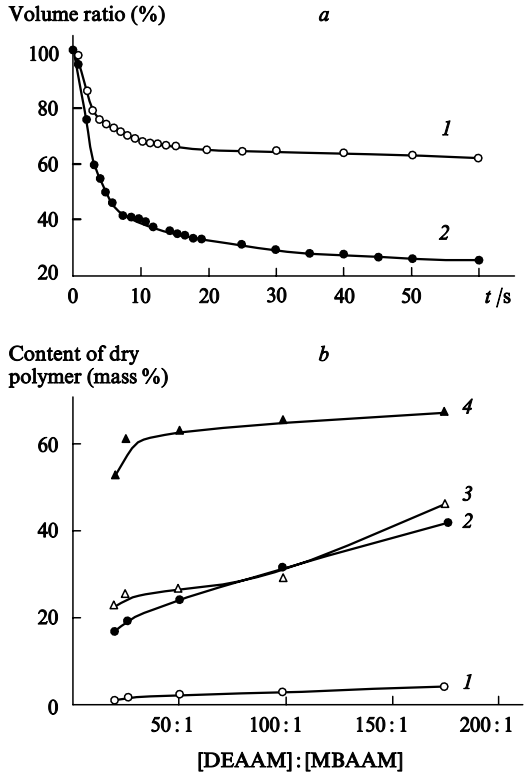


Figure 7. Properties of PDEAAM gels and cryogels.[192] (*a*) dynamics of the thermally induced collapse of the PDEAAM gel (*1*) and cryogel (*2*); (*b*) content of the dry polymer in the gel phase at temperatures below (20 °C, curves *1*, *3*) and above (60 °C, curves *2*, *4*) the point of thermally induced collapse of the PDEAAM gel (*1*, *2*) and cryogel (*3*, *4*) for specimens with different initial ratios of the vinyl and divinyl comonomers and a constant total concentration (5.66%).

Unlike polymerisation cryogels, polycondensation analogues have been little studied, although actually, the only restriction hampering the synthesis of the latter may be the fact that many polycondensation reactions giving the corresponding polymers are endothermic and require heating of the monomer solution. In the case where the synthetic route chosen uses reactions with positive heats and the reactivity of monomers is rather high, the formation of polycondensation cryogels almost does not differ from the formation of polymerisation cryogels.

In particular, freezing is known [194–199] to exert a structuring effect on hydrated sols of weak inorganic hydroxides (for example, iron hydroxide). The transformation of these sols into gels follows a polycondensation mechanism with water evolution to give a three-dimensional network of the inorganic oxide. The formation of silica gel on ageing of silicic acid sols is known [200, 201] to proceed in a similar way; therefore, the cryogenic variant of this process can be regarded as polycondensation cryotropic gelation. Freezing of a silicic sol which liberates water during a chemical reaction, *i.e.*, forced dehydration of the system, should apparently shift the equilibrium towards the final product.

Preparation of silica cryogel is not an easy experimental task,[22] because it requires a rather accurate acid titration of an alkaline solution of a silicate with cooling until a silicic sol is formed. Then the colloid system must be rapidly frozen to ensure that polycondensation (*i.e.*, ageing of the sol) occurs mainly in the NFLMP of the macrofrozen specimen. An interesting feature of the formation of silica cryogel is the fact that after thawing, the inorganic gel is obtained as scales rather than as a lump. The size of particles (2–5 mm) depends on the temperature of the cryogenic treatment. This is due to the fragility of silica cryogel, which is crushed by the growing ice crystals. In the preparation of elastic cryogels based on organic polymers, sample destruction of this type is hardly ever observed.

The structure and properties of silica cryogels are mainly determined by the concentration of reactants and the temperature of freezing (Table 3). In particular, when the initial silicate concentration is above 0.5 mol litre^{-1}, the specific surface areas of the usual silica gel and silica cryogels formed at −10, −20, and −30 °C almost do not differ. However, cryogenic treatment of less concentrated systems affords silica cryogels whose specific surface areas are greater than that of the silica gel prepared from the reaction mixture with the same composition at a positive temperature. Silica cryogels formed at −10 °C possess a greater specific surface area than the specimens prepared at −20 and −30 °C. Unfortunately, the experimental data concerning inorganic cryogels of this type available to date are inadequate for a well-posed interpretation of this trend.

Table 3. Dependence of the specific surface area of silica gels and silica cryogels on the SiO_2 concentration in a silicic acid sol and on the gelation temperature.[22]

Concentration of SiO_2 in the silicic acid sol /mol litre^{-1}	Specific surface area ($m^2\ g^{-1}$) of the dry material formed at different temperatures			
	20 °C	−10 °C	−20 °C	−30 °C
0.7	90	102	96	94
0.5	350	375	370	360
0.4	510	600	550	530
0.3	590	750	690	645
0.25	630	875	810	740

[a] Determined from adsorption of argon.

To the best of the author's knowledge, there are no publications dealing with polycondensation cryogels based on organic polymers. These cryogels are still to be prepared and comprehensively studied.

2. The formation of cryogels in solvent – polymer – cross-linking agent systems

Cross-linking in solutions of macromolecular precursors on treatment with appropriate cross-linking reagents has long been known and is widely used both in the laboratory and in industry for the preparation of lyogels. Moderate freezing of initial systems of this type, incubation in the frozen state for a required period of time and subsequent thawing lead to macroporous cryogels.

It is notable that cryostructurates formed by cross-linking of polymer precursors in frozen media were known a long time ago. Indeed, the porous foodstuff 'kori-tofu', rather popular in Japan for several centuries, is nothing more than a cryogel based on soya proteins (so-called 11S-globulins) with covalent cross-linking by intermolecular disulfide bonds.[202] Moreover, cross-linking of polypeptide chains at cysteine residues takes place just during the cryogenic treatment.[203, 204] Using model thiol-containing polymers (SH derivatives of carboxymethylcellulose and PAAM), it has been shown [205] that atmospheric oxygen dissolved in the aqueous medium acts as the oxidant in these systems.

Thiol-containing water-soluble polymers proved to be very convenient objects for the study of the kinetics of cryotropic gelation of this type [83, 206] because the course of the reaction can be monitored by highly sensitive spectrophotometric titration of the SH groups. Figure 8 shows the time variation of the concentration [calculated as the ratio of the current to initial SH titres expressed in per cent (Q_{rel})] of sulfhydryl groups in liquid and frozen aqueous solutions of the thiol derivative of PAAM at various temperatures. It can be seen that in frozen specimens (at −15 and −20 °C), the concentration of the SH groups decreases much faster than in liquid solutions; this markedly accelerates cryo-

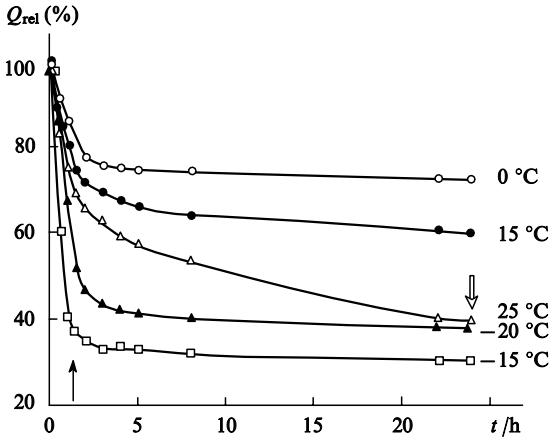


Figure 8. Kinetics of gelation of the SH derivative of PAAM at various temperatures in a liquid and frozen aqueous solution of the polymer (concentration 2 g dl^{-1}, pH 7.8).[22, 83]

tropic gelation. For example, at −15 °C, a cross-linked gel is formed over a period of approximately 1 h (shown by the black arrow), whereas at 25 °C, this occurs only after 24 h (light arrow). In other words, for the cryostructuring of solvent–polymer–cross-linking agent systems, as for the formation of polymerisation cryogels, gelation in a definite range of negative temperatures is accelerated compared to that for non-frozen specimens.

At −5 °C, an aqueous solution of SH-PAAM with a polymer concentration of 2 g dl^{-1} does not become frozen due to undercooling effects; therefore, the reaction is very slow under these conditions. When low-temperature quenching is used, the system is frozen at −5 °C and the reaction rate is high owing to cryoconcentration (Fig. 9). The temperature dependence of the rate of variation of Q_{rel} in systems frozen by two different procedures passes through an extremum, which is typical of cryotropic gelation. Comparison of the pattern of variation of the SH-group concentration with the NMR data on the amount of mobile non-frozen solvent in the specimens shows [83] that after a specified negative temperature has been attained, the volumes and properties of liquid microphases in the specimens frozen in different ways are non-equivalent for a certain period of time. In the case of freezing from above, after crystallisation of the main bulk of the solvent, the reactions proceed initially under conditions of gradually decreasing volume of the liquid component, *i.e.*, with increasing concentrations of reactants. Conversely, in the specimens frozen in liquid nitrogen and then heated to a specified negative temperature, the smallest volume of the microreactor and, hence, the greatest degree of substance concentration is attained almost simultaneously when the temperature of the environment (the cryostat refrigerating fluid) becomes equal to the temperature of the frozen reaction mixture. These dynamic differences between the phase states of the specimens brought to the temperature of a cryochemical reaction *via* different routes account for the non-equivalence of temperature dependences *1* and *2* in Fig. 9.

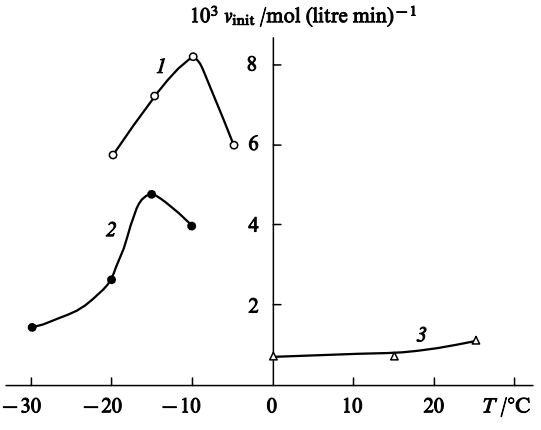


Figure 9. Initial rate (v_{init}) of gelation of the SH derivative of PAAM *vs.* temperature (concentration 2 g dl^{-1}, pH 7.8).[22, 83]
(*1*) Low-temperature quenching, (*2*) freezing from above, (*3*) variation of v_{init} for the region of positive temperatures.

Cryotropic gelation in the solvent–polymer–cross-linking agent systems, like the synthesis of polymerisation cryogels, is accompanied by an apparent decrease in the CCG. For example, Fig. 10 shows the dependences of the yield of the gel fraction on the polymer concentration in the initial solution (Fig. 10 *a*) and on the amount of the cross-linking agent (Fig. 10 *b*) during the formation of normal gels and cryogels of chitosan [poly-β-(1 → 4)-D-glucosamine] cross-linked by glutaraldehyde at 25 °C and in a moderately (−8 °C) frozen medium. Cryogels are formed at substantially lower initial concentrations of both the polymer (Fig. 10 *a*) and the cross-linking agent (Fig. 10 *b*).[13, 22] Undoubtedly, this decrease in the CCG is due to the cryoconcentration of the reactants in the NFLMP.

The normal gels of cross-linked chitosan are typical homophase hydrogels; in the swollen state, they are transparent and rather brittle, especially when the cross-linking degrees are relatively high. The whole bulk of the solvent present in the specimens swollen to equilibrium occurs in the bound (solvation) state. As in the case of other chemically cross-linked gels, it cannot be removed by squeezing out without disruption of the gel integrity. Conversely, cryogels formed from the same initial reaction solutions are heterophase spongy non-transparent materials in which the capillary-bound solvent can be separated rather easily by compression even at low mechanical loads.

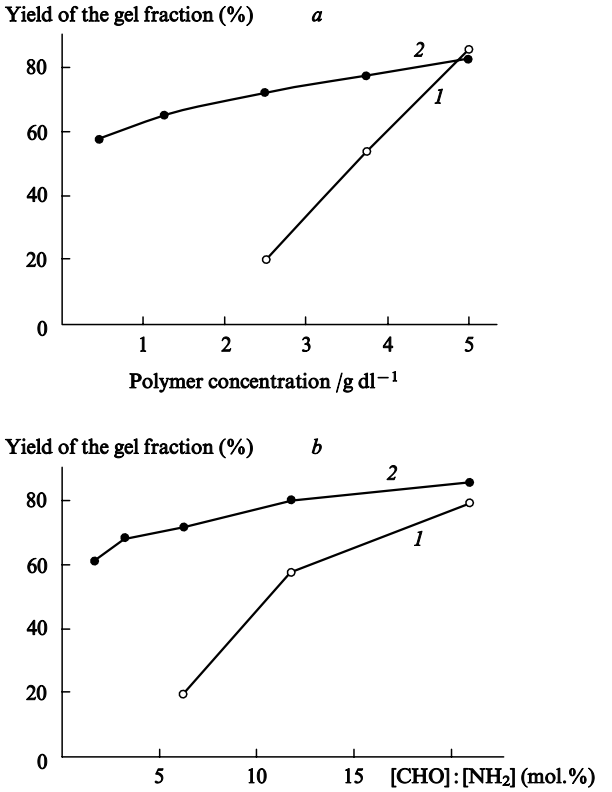


Figure 10. Yield of the gel fraction *vs.* the initial concentration of chitosan (*a*) and *vs.* the ratio of the number of glutaraldehyde CHO groups to the number of polymer amino groups for a fixed (2.5 g dl^{-1}) initial concentration of chitosan (*b*) [13, 22] at (*1*) 25 °C, (*2*) −8 °C.

The above examples refer to the synthesis of cryogels in frozen aqueous media. However, there are no obstacles preventing, in principle, the preparation of cryogels from precursors soluble in organic solvents. A necessary condition is that the organic solvent should undergo crystallisation rather than vitrification to ensure the separation of the solid and liquid phases and the formation of an NFLMP with an enhanced concentration of reactants.

Figure 11 shows the dependence [22, 30] of the yield of the gel fraction on the polymer concentration in the initial solution (Fig. 11 *a*) and on the temperature (Fig. 11 *b*) during the $SnCl_4$-catalysed cross-linking of polystyrene with 1,4-xylylene dichloride according to the Friedel–Crafts reaction in liquid (13.5 °C) and crystallised (−13.5 °C) nitrobenzene (T_m = 5.5 °C). These data indicate that processes in organic solvents actually obey the same regularities as gelation in aqueous media, namely, the apparent decrease in the CCG for cryogenic processes (Fig. 11 *a*) and an extremal pattern of dependence of their efficiency on the temperature of the frozen system (Fig. 11 *b*). The polystyrene cryogels formed in crystallised nitrobenzene have a spongy morphology (similarly to cryogels based on hydrophilic polymers considered above) and during swelling absorb substantial amounts of organic solvents compatible with polystyrene, for example, benzene or toluene. The main bulk of the liquid absorbed by the cryogel is the capillary solvent which can be easily squeezed out. Thus, it can be concluded that the type of morphology of cryogels cross-linked by covalent bonds and the effects involved in their formation are rather general and do not depend on the nature of the crystallisable solvent used.

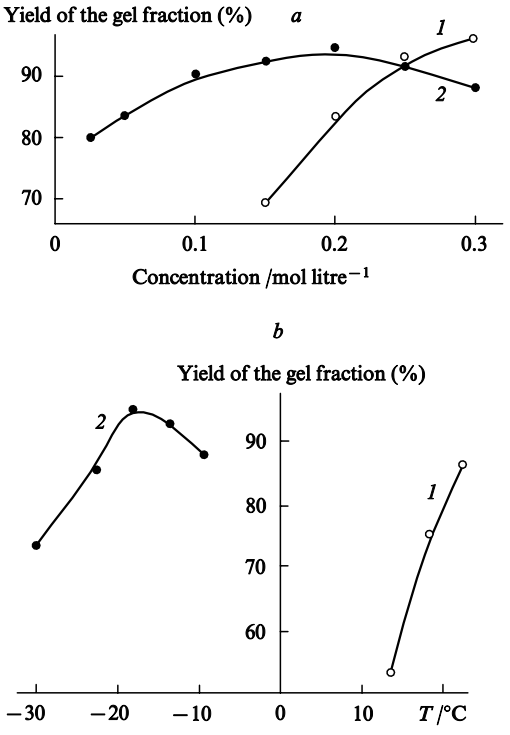


Figure 11. Yield of the gel fraction *vs.* the initial concentration of polystyrene expressed as the molar concentration of monomer units (*a*) and *vs.* the temperature (the initial concentration of monomer units of polystyrene is 0.3 mol litre^{-1}) (*b*);[22, 30]
(*1*) formation of gels (13.5 °C); (*2*) formation of cryogels (−13.5 °C).

3. Ionic cryogels

Ionic, coordination, and coordination–ionic polymer gels represent special types of gel-like systems. On the one hand, they are thermally irreversible, like covalently cross-linked gels, and on the other hand, the change in the pH or the salt composition of the liquid which surrounds the specimen can induce fast dissolution of swollen ionotropic and chelatotropic networks, which are converted again into the gel upon an appropriate change in the medium composition; this reversibility makes these systems similar to physical gels. A specific feature of many ionotropic gels is heterogeneity caused by impediments to the diffusion of new portions of counter-ions into deep regions of the gelling specimen through the gel membrane, which is formed rapidly at the sample surface (periphery).

Traditional ionotropic gels, *i.e.*, those formed at positive temperatures, can be divided conventionally into three groups.

The first group includes solvated networks based on polyelectrolytes whose macromolecules are cross-linked to give a three-dimensional structure through low-molecular-weight ions forming low-dissociated salt bridges with the ionogenic groups of the polyelectrolyte chains at the network junction knots. When complexing agents are used for cross-linking, a chelate (coordination) network is formed. These ionotropic and chelatotropic gels are capable of substantial repeated swelling after drying.

The second group includes polymer gels formed with the change in the charge of polyelectrolyte ionogenic groups, resulting in a decrease in the polymer solubility and phase separation. The gel-like systems formed in this case usually possess low mechanical strengths and, after drying, they are unable to swell to a substantial extent in the same medium as was used for gelation.

The third group comprises ionotropic gels formed during preparation of polyelectrolyte complexes (PEC). In essence, these systems represent swollen three-dimensional networks cross-linked by poorly dissociating salt bonds (similarly to the first-group ionic gels). However, in this case, it is often impossible to establish which of the PEC components (either polycation or polyanion) is the gel-forming and cross-linking component, especially if the MW and the initial concentrations of these compounds are similar.

Examples of preparation of ionotropic and chelatotropic cryogels that can be attributed (although with some reservations) to the first two groups have now been reported. Cryogels based on PEC have not yet been described.

The major obstacle faced during the formation of ionotropic cryogels is a too high rate of gelation, which proceeds even at the stage of component mixing. Indeed, if low-molecular-weight counter-ions inducing gelation are added to a polyelectrolyte solution in a concentration higher than the CCG, a lyogel is formed very rapidly. On subsequent freezing, the solvent crystallises in the gel phase, *i.e.*, it is virtually impossible to freeze the system when it still exists in the liquid state. This situation is encountered, for example, in the attempt to prepare a calcium alginate cryogel: the addition of a solution of a calcium salt to an aqueous solution of sodium alginate (the sodium salt of the linear block copolymer of D-mannuronic acid and L-guluronic acid) entails fast formation of a gel network, and the system loses fluidity. This specimen can be frozen, but this would not be accompanied by accumulation of precursors and by chain cross-linking in the NFLMP, peculiar to cryotropic gelation (Fig. 12, pathway *a*). Preparation of the cryogel by recharging the alginate carboxy groups is impossible either, because acidifying the solution results in fast formation of a thixotropic gel of alginic acid, whose freezing–thawing does not afford a macroporous material typical of cryogels (Fig. 12, pathway *b*).

Two approaches have been proposed to surmount these obstacles (Fig. 12, pathways *c* and *d*).

If a finely powdered salt having a negative temperature factor of solubility is added (as a source of cross-linking counter-ions) to a polyelectrolyte solution (Fig. 12, pathway *c*) and the resulting suspension is subjected to moderate freezing, gradual dissolution of this salt in the NFLMP and cross-linking of the polyelectrolyte chains take place at low temperature. After thawing, a macroporous ionotropic cryogel is produced. A crucial point is the choice of an appropriate salt because the solubility of most salts increases with increasing rather than decreasing the temperature.

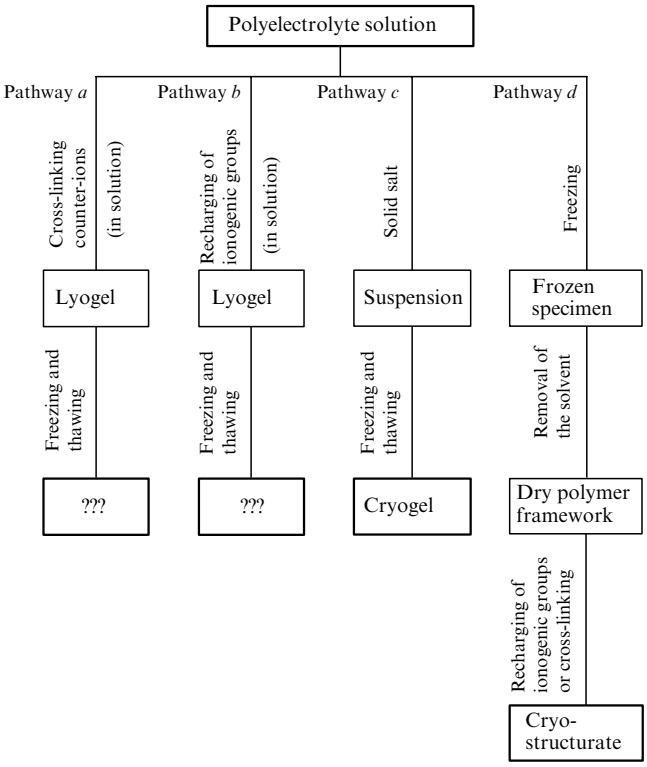


Figure 12. Basic diagram of the formation of ionic cryostructurates.[22]

The degree of salt grinding is also significant. This strategy has been employed to prepare an alginate cryogel[22, 207, 208] using calcium salts with the solubility increasing upon decreasing temperature [in particular, calcium butyrate, pentanoate, succinate, glycerophosphate, and some other calcium salts have negative temperature factors of solubility in water (see Ref. 209, p. 1353)]. In this case, as in the formation of covalent cryogels, the properties of the final material depend not only on the precursor concentrations and the temperature of cryogenic treatment but also on the time of keeping the specimens in the frozen state. In particular, when the initial concentrations of sodium alginate and calcium glycerophosphate are 0.5 mass %, keeping frozen specimens at -10 °C for 1.5 h gives ultimately (*i.e.*, after thawing, washing and drying) a cryogel with a rupture strength of 250 kg cm^{-2}. An increase in the duration of the cryogenic treatment to 3 h leads to a material with a rupture strength of 420 kg cm^{-2}, and incubation of the sample in the frozen state for 24 h makes this parameter about twice as high (800 kg cm^{-2}).[22, 208] These data indicate that cross-linking and structuring of alginate require a certain time for dissolution of the solid cross-linking agent and for slow diffusion of calcium ions into the high-viscosity medium of the liquid microphase.

However, this strategy for the preparation of ionotropic cryogels is subject to numerous restrictions, related, most of all, to the admissible concentrations of precursors. Above a definite concentration of either the polyelectrolyte or the salt, too fast gelation taking place at positive temperatures prevents the formation of a 'true' cryogel.

Almost no limitations of this type are involved in the procedure shown as pathway *d* in Fig. 12. This strategy allows the preparation of macroporous ionotropic cryogels by both the recharge of ionogenic groups and cross-linking with appropriate counter-ions, although cryotropic gelation does not actually occur in this case.[22, 210, 211] To prepare the target material, a polyelectrolyte solution with any desired concentration limited only by the polymer solubility is merely frozen at a required temperature. This is followed by removal of the crystallised solvent without thawing, for example, by vacuum sublimation (freeze-drying) or cryoextraction (treatment at a negative temperature with a liquid which acts as a solvent for the crystalline phase but a non-solvent for the polyelectrolyte). Finally, cryogenically structured macroporous polymeric framework is obtained, which is then transformed into an insoluble state either by treatment with cross-linking counter-ions or by recharging ionogenic groups. The last-mentioned operation is also carried out in the polymer non-solvent and this no longer requires low temperatures.

For instance, freezing of a solution of sodium alginate and freeze-drying of the specimen thus formed followed by immersion of the porous material into an ethanol solution of calcium acetate results in an ion exchange reaction in which the material structure is fixed by poorly dissociating salt bridges. This yields a calcium alginate cryostructurate[210] which is not dissolved in a physiological salt solution for several days, whereas a usual alginate gel prepared from the same precursors at a positive temperature loses its integrity in several minutes and is promptly dissolved. When substances capable of forming poorly dissociating coordination (more precisely, coordination – ionic) bonds with alginate groups, for example, chromium salts, are used as cross-linking agents, this procedure leads to chelatotropic cryostructurates.[22]

Treatment of plates or grains of a frozen solution of sodium alginate with acidified acetone in the cold (*e.g.*, at -20 °C) induces cryoextraction of ice with simultaneous transformation of the polymer network into alginic acid due to recharge of ionogenic groups. The final macroporous polymer is insoluble in water but is wetted by water and readily swells.[210]

A similar strategy can be utilised to prepare a macroporous material from a polymeric base. A specimen prepared by freezing a solution of a macromolecular salt followed by removal of the solvent is treated, for recharge of ionogenic groups, with an alkaline reagent in a medium that is a non-solvent for the polymer.[211] For example, dropwise addition of a solution of chitosan in dilute acetic acid to cooled (*e.g.*, to -15 °C) octane results in frozen spherical particles, treatment of which with a saturated solution of NaOH in acetone or methanol at a negative temperature affords granulated macroporous chitosan base. This material is stable in alkaline or neutral media and is capable of coordinating heavy metal ions (for example, its capacity with respect to Cu^{2+} or Co^{3+} ions equals 5.2 – 5.5 mmol g^{-1}).[22, 211]

Thus, it is possible to carry out cryogenic structuring of polyelectrolytes, although it requires more sophisticated approaches, and to prepare ionic or coordination polymer materials possessing macroporous morphology, typical of cryogels.

4. Cryogels with a physical network of the polymer phase

As has been noted in the Introduction, non-covalent (physical) cryogels are the best known and investigated gel materials formed by the freezing – thawing strategy. This refers most of all to PVA cryogels. A detailed analysis of publications (by the end of 1997) devoted to the formation of PVA cryogels, the influence of cryogenic treatment parameters on these gels, and their properties has been given in a review.[9] In this Section, we consider only the general fundamental items of non-covalent cryotropic gelation and the data on physical cryogels based on other polymers. However, at the end of the Section, some other results concerning PVA cryogels (published in 1998 – 2001) are also considered.

Generally, non-covalent physical gels form a very extensive class of gel systems produced in both aqueous and organic media (see, for example, the publications[15, 20, 212]). In principle, non-covalent gels can be prepared by either cooling (if the solvent – polymer system has an UCST) or heating (if the system has an LCST). In the former case, one deals with psychrotropic gels, and in the latter case, thermotropic gels are involved. It is clear that the latter cannot be cryogels. But in the case of psychrotropic gel-forming systems, it is not always possible to prepare a physical cryogel by mere freezing. When the polymer concentration is above the critical point and the rate of material solidification on cooling is high, then, as in the case of polyelectrolytes with cross-

linking counter-ions (Section IV.3), fast gelling takes place. In this case, the system that undergoes freezing is no longer a solution but a gel; therefore, the structure of the polymeric material after thawing differs from that obtained in the case of 'standard' cryotropic gelation. An example of such a system is an aqueous solution of agarose (neutral polysaccharide, the major component of agar-agar), which is converted into a gel over a period of several minutes even at 40–30 °C when the polymer concentration is above 1%. An agarose macroporous cryogel can be prepared only from relatively dilute (< 0.2%) solutions of the polymer. However, strength characteristics of such a cryogel are poor.

Solutions of PVA (in water or DMSO),[9, 31–34, 213] amylopectin,[214] maltodextrin (a mixture of partially depolymerised starch polysaccharides with MW < 20 kDa),[215] starch,[23, 216–219] or LBG [23–26] are more convenient for the formation of non-covalent cryogels because they belong to systems gelling rather slowly on decreasing the temperature. They can be frozen without complications, stored for a required period at a specified negative temperature and then thawed to give the corresponding cryogels. The cryogels based on these polymers are thermally reversible: they fuse at elevated temperatures and repeated freezing–thawing of the solution results again in a cryogel.[22, 31, 220]

Depending on the type and initial concentration of the polymer and the regimes of cryogenic treatment, this gives either spongy cryostructurates with moderate strength similar to cryo-PAAG, described in Section IV.1, or elastic non-spongy cryogels (which are exemplified by PVA cryogels). Most likely, these distinctions in the morphology of materials are related to the amount of free (unbound) solvent able to crystallise at a chosen negative temperature and to the size of porogen polycrystals thus formed. In some cases, the same solvent–polymer system with different contents of the macromolecular component can be converted in both spongy and non-spongy cryogels.

For example, dilute (0.05–0.75 g dl^{-1}), semidilute (0.75–2.0 g dl^{-1}) and concentrated (> 2.5 g dl^{-1}) aqueous solutions of LBG remain liquid at room temperature for several months.[221] Freezing–thawing (−10 to −30 °C) of these solutions gives very weak spongy cryostructurates in the first case, good polymeric sponges in the second case, and elastic non-spongy cryogels in the third case.[23–26]

Aqueous solutions of maltodextrin containing less than 15–20 g of the polymer per dl (the concentration depends on the MW) cannot be transformed into a gel without cryogenic treatment. Concentrated aqueous solutions of PVA containing no residual acetyl groups form gels very slowly, whereas the freezing–thawing procedure always gives cryogels.[9, 22, 31–34, 213, 215] Thus, thermally reversible physical cryogels are obtained at lower initial concentrations of the macromolecular precursors and often much faster than without freezing. This indicates that non-covalent cryotropic gelation, like the formation of covalently cross-linked cryogels, is subject to the effects of the apparent decrease in the CCG and accelerated gelation.

The chemical nature of the intermolecular bonds stabilising the junction knots of the three-dimensional polymer network in physical cryogels depends on the structure of the gel-forming polymer material. Usually, these are either hydrogen bonds (the predominant type of contacts between the chains in thermally reversible cryogels, similar to cryogels based on PVA, starch, and other polyhydroxy polymers) [222–224] or hydrophobic interactions, which play an important role in the formation of new protein cryogels,[82, 225] or various weak (but cooperative) dispersion interactions. For PVA cryogels, the formation of microcrystallinity zones acting as junction knots in the supramolecular network has been shown experimentally.[226–228] The sizes of microcrystallites are determined by the temperature conditions of cryostructuring and the number of freezing–thawing cycles. At these knots, syndiotactic sites of PVA chains form numerous hydrogen bonds.[9, 22] Apparently, microcrystallinity zones function as the physical network junction knots also in the structure of cryogels formed from starch, maltodextrin, and LBG polysaccharides.

The macro- and micromorphology and physicomechanical and thermal properties of non-covalent cryogels depend appreciably on some characteristics of the polymer precursors, which are not so important in the case where cryogels are formed through covalent cross-linking of macromolecules. For example, in the cryotropic gelation of PVA solutions, apart from essential properties of the polymer such as the MW and chain tacticity, the number of residual *O*-acyl groups [*O*-acetyl groups for the PVA prepared from poly(vinyl acetate)] is also a significant factor, as the presence of even ~5 mol. % of these substituents is sufficient for inhibiting the complementary ('side-by-side') interaction of neighbouring chains.[213, 229–232] In other words, *O*-acyl groups act as sort of distance bars preventing the formation of numerous intermolecular hydrogen bonds and thus interfering with the formation of PVA cryogels. In the case of starch cryogels, an important parameter is the ratio of the linear to branched polysaccharides (amylose and amylopectin) in the system subjected to freezing, which depends on the type of starch used and, in some cases, on the procedure of its pretreatment.[23, 214–219, 233–236] When non-covalent cryogels such as albumin cryogels are formed through hydrophobic interactions, the accessibility of hydrophobic regions for intermolecular contacts becomes the crucial factor. This is determined by the conformation of chains, in particular, by denaturation-related changes in the macromolecules of these globular proteins, which are specially induced by pretreatment (prior to freezing) such as the addition of chaotropic agents (which destroy the cluster structure of water), heating, or high-pressure treatment.[82, 237–243] Thus, as in the case of 'usual' psychrotropic gelation, for the formation of physical cryogels, too, the chemical structure and the degree of exposure of the chain groups involved in intermolecular interactions are important factors.

The conditions of all stages of cryogenic treatment are highly significant for the objects considered. Whereas in the synthesis of cryogels cross-linked by covalent or ionic bonds, the conditions of thawing of specimens usually have little influence on the properties of final gels, the parameters of non-covalent cryogels are often fairly sensitive to the thawing conditions.[9, 10, 14, 26, 31–34, 214, 215, 235, 244]

For example, Fig. 13 shows the variation of the apparent instantaneous modulus of elasticity (E_0) and the fusion temperature (T_f) of the LBG cryogels *vs.* the thawing rate of the specimens (the v_{th} values are laid-off along the abscissa axis on a logarithmic scale).[22, 26] The specimens were prepared by freezing aqueous solutions of a polysaccharide (2.0 g dl^{-1}) at −20 °C for 18 h. It can be seen that the lower the thawing rate, the greater the rigidity and the thermal stability of these physical cryogels. This trend is typical of those thermally reversible cryogels in which hydrogen bonding is the predominant type of intermolecular contacts stabilising the polymer network junction knots (cryogels

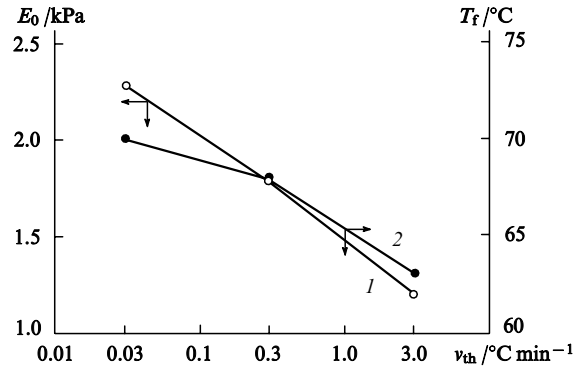


Figure 13. Properties of LBG cryogels *vs.* v_{th}.[22, 26]
(*1*) Apparent instantaneous modulus of elasticity, (*2*) cryogel fusion temperature.

based on PVA, LBG, starch polysaccharides, gelatin, agar-agar, *etc*., are examples of such systems).

The nature of this effect has been studied in most detail for PVA cryogels.[9, 22, 31, 33, 34, 80, 244, 245] The data of Table 4 indicate that the shear modulus (G_0) of PVA cryogel specimens prepared from aqueous solutions with different polymer concentrations increases severalfold as the thawing rate decreases from 0.3 to 0.03 °C min^{-1}.[34, 245] Conversely, in the case of high thawing rates (> 5–10 °C min^{-1}), PVA cryogels cannot be prepared at all, as, instead of an elastic gel, thawing gives a muddy thick liquid.[31, 33, 34] These data indicate that the formation of PVA cryogels takes place exactly at the thawing stage. One can follow the kinetics of gelation and choose the temperature range corresponding to the highest intensity of this process (Fig. 14) using the following procedure: aqueous solutions of PVA are first frozen, for example, at −20°C, then the specimens are transferred into a cryostat with a specified subzero temperature and, after incubation over particular periods of time, selected specimens are rapidly thawed (at a rate of ~3 °C min^{-1}) in order to eliminate the effect of the thawing rate.[22, 34]

Table 4. Effect of the thawing rate on the rheological properties of PVA cryogels.[22, 34, 245]

Concentration of PVA in the initial solution /g dl^{-1}	Rate of thawing of frozen specimens /°C·min^{-1}	Shear modulus of PVA cryogel specimen /kPa
3.0	0.30	< 0.3
3.0	0.03	< 0.3
5.0	0.30	< 0.3
5.0	0.03	1.73
7.0	0.30	1.80
7.0	0.03	3.93
10.0	0.30	4.94
10.0	0.03	10.6
10.0 [a]	3.0	1.40
10.0 [a]	0.30	3.20
10.0 [a]	0.03	9.40

Note. The materials were prepared by freezing at −20 °C for 18 h a completely deacetylated PVA with MW of 115 kDa. [a] PVC deacetylated to 99% with MW of 69 kDa.

The 0-h points in the plots correspond to the onset of incubation of frozen specimens at a specified subzero temperature (which is written near the corresponding curves in Fig. 14 *a*). Figure 14 *b* shows the duration of this incubation. The shear modulus of the PVA cryogels prepared using this technique increases rather weakly whilst keeping the frozen specimens at −10 °C. At higher negative temperatures, gelation becomes much more intense. The observed temperature dependence is non-monotonic, the process being faster in the −2 to −3 °C range than at −1.5 °C. Thus, in this case, the regularities are qualitatively analogous to the features of covalent cryotropic gelation during the synthesis of cryogels by polymerisation of monomer precursors (see Fig. 5) or by chemical cross-linking of polymer precursors (see Fig. 8 *b* and Fig. 11 *b*), *i.e*., the temperature dependence of the efficiency of formation of non-covalent PVA cryogels at negative temperatures passes through an extremum.

Study of frozen solutions of PVA by ESR and NMR spectroscopy shows [70, 81] a sharp increase in the amount of the mobile solvent above a certain threshold temperature range, which is 4–6 °C below the crystallisation point of the system. At these temperatures, together with firmly bound non-freezable water, an additional amount of liquid water appears in the solid, frozen specimen. This partial melting of ice increases the NFLMP volume; therefore, the mobility of macromolecules (or their segments) and the efficiency of polymer–polymer interactions

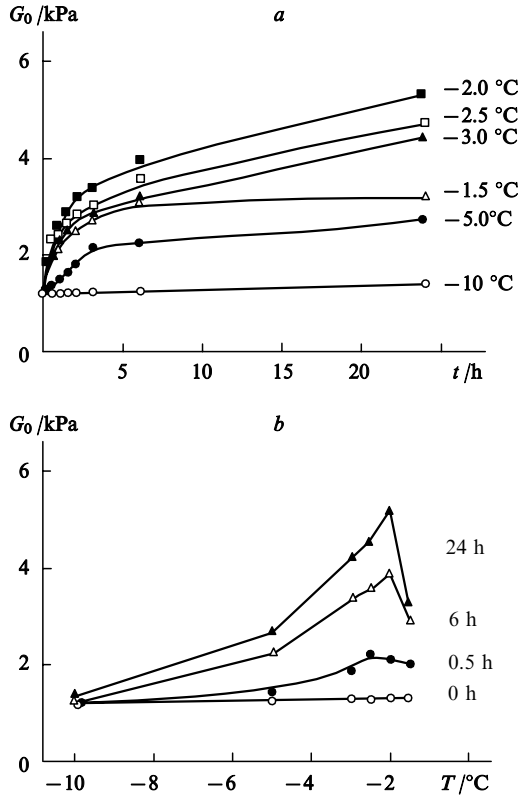


Figure 14. Dynamics of formation of PVA cryogels (completely deacetylated PVA with MW of 115 kDa, initial concentration 7 g dl^{-1}, freezing at −20 °C over a period of 24 h) at subzero temperatures.[22, 34]
(*a*) Time variation of rigidity of PVA cryogels; (*b*) temperature dependences of the rigidity of specimens kept in the subzero region.

increase. Finally, this results in the formation of microcrystallites which act as junction knots of the cryogel network. In particular, this mobility can be detected in ^{13}C NMR spectra: ~8 °C below the melting point of the main bulk of the solvent, the resonance peaks for the carbon atoms of PVA virtually cannot be detected but when the temperature rises by ~3 °C, quite resolved, although weak signals arise. On further increasing the temperature (2–3 °C prior to thawing of the specimen), a real ^{13}C NMR spectrum for the polymer solution is recorded. From this it follows that a temperature 5–6 °C below the system's thawing point corresponds to a boundary of retardation of the fast (on the NMR time scale) motions of the PVA carbochain backbone. Estimation of the spin–lattice relaxation correlation times for the carbon nuclei in the polymer, which serve as the criterion for the mobility of kinetic chain segments, shows that reorientation of the ^{13}C nuclei in the PVA methylene groups in a system frozen at subzero temperatures occurs ~100 times more slowly than that in the initial liquid solution. This points to a very high viscosity of the NFLMP.[81]

On the basis of these data, it is possible to interpret the influence of the thawing rate or the duration of incubation of frozen specimens at moderate negative temperatures on the properties of PVA cryogels (see Fig. 14). The slower the thawing and the closer its conditions to equilibrium, the longer the period the specimen is at the temperatures optimal for gel network formation. Therefore, slow warming-up during thawing of the preparations is equivalent, in a sense, to storing the specimen for a long period at temperatures where the strongest cryogels are formed. Although a high polymer concentration in the NFLMP is favourable for gelation, this requires a fairly long period of time due to substantial viscosity of the NFLMP and low mobility of chains. The extremal dependence of the physicomechanical properties of PVA cryogels on the cryostructuring temperature (see

Fig. 14 *b*) is due to the competition of factors favourable and unfavourable for gel formation. On the one hand, at temperatures above the extremum point, the amount of mobile solvent is greater, *i.e.*, the specific concentration of the gel-forming reagent in the NFLMP is lower, so is the probability of formation of effective intermolecular contacts (intensification of thermal motions in the system also counteracts stabilisation of the contacts). On the other hand, at temperatures below the extremum, the amount of mobile solvent decreases, the polymer becomes poorly solvated and retains only the firmly bound water, *i.e.*, the liquid medium in which the motion bringing together the interacting sections of the neighbouring chains takes place is no longer present.

Since concentrated aqueous solutions of PVA usually do not freeze in the subzero temperature range, *i.e.*, at temperatures of most intense gelation, due to undercooling effects, the initial specimens always need to be frozen at lower temperatures. The properties of PVA cryogels can be varied by varying the rate of thawing or the time of storage of the frozen materials at subzero temperatures.

Figure 15 shows the pattern of porosity of specimens of PVA cryogels thawed at rates of 0.3 and 0.03 °C min^{-1}. The photomicrographs of thin sections of cryogels dyed by Congo Red were taken using optical microscopy; the dark areas represent the gel phase and the light areas are macropores.[22, 245] Whereas in the first specimen (Fig. 15 *a*), the macropore size and arrangement are rather irregular, the specimen of the PVA cryogel formed under slow thawing conditions (Fig. 15 *b*) possesses quite a perfect morphology with a clear-cut alternation of oblong macropores and strands of the gel phase. Undoubtedly, higher rigidity of slowly thawed cryogels of PVA of this type compared to the specimens thawed more rapidly (see Table 4) is due, among other factors, to the more regular macrostructure of the former.

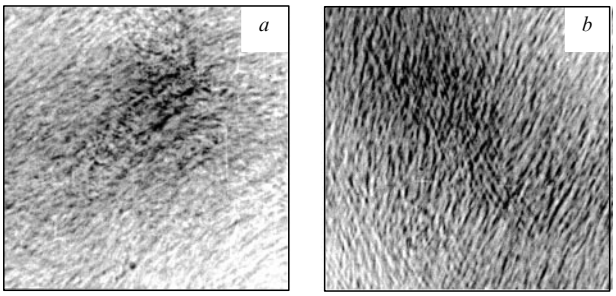


Figure 15. Photomicrographs (optical microscope) of thin sections of specimens of PVA cryogels formed from an aqueous solution (12 g dl^{-1}) of a polymer (MW 69 kDa, degree of deacetylation 99 mol.%) on freezing at −20 °C for 18 h followed by thawing at a rate of 0.3 (*a*) or 0.03 °C min^{-1} (*b*) (see Refs 22, 34). Scale: 40 μm to 1 cm.

It is worth noting that the morphology of PVA cryogels has been studied most often by electron microscopy techniques, which imply dehydration of specimens,[4, 22, 226, 246–253] resulting inevitably in some changes in the dimensions and shapes of the structural units of these objects.[246, 249, 250, 253] The use of optical microscopy for these studies is more legitimate because it permits one to observe the real structure of a swollen cryogel.[226, 245, 253–255]

Yet another interesting feature of non-covalent cryogels is an increase in the rigidity upon freezing and thawing repeated many times.[4, 5, 9, 11, 25, 32, 226–228, 230–232, 256–258] For example, the dynamic modulus of elasticity of a PVA cryogel prepared from a 15% aqueous solution of the polymer (MW 75 kDa) is an order of magnitude higher after four cycles of cryogenic treatment (each cycle includes 24 h freezing at −20 °C and 7 h thawing at 15 °C) than after one such cycle.[259] According to the data of X-ray diffraction analysis and DSC,[226–228, 260] every subsequent freezing–thawing procedure increases the content and the size of crystallites in the PVA cryogel. Apparently, repeated occurrence of the system in the temperature of the most intense gelation gives rise to new additional intermolecular contacts and extends those present previously. This is manifested, in particular, as numerous thickened regions observed through an optical microscope.[226] In addition, the possible compaction of the supramolecular strands of the cryogel network, which approach one another under the action of growing ice crystals, cannot but be taken into account.

Thus, various procedures of cryogenic treatment of the water–PVA system make it possible to vary the properties and morphology of non-covalent cryogels of a given polymer over wide limits,[9, 88, 245, 261–268] the duration of the occurrence of the frozen specimen at moderate negative temperatures being the crucial factor determining the efficiency of non-covalent cryotropic gelation.

This conclusion is also true for the formation of other physical cryogels. An important role of thawing or incubation at subzero temperatures was demonstrated for the cryotropic gelation of LBG,[26] maltodextrin,[215] amylopectin,[214] and mixtures of amylopectin with amylose.[235] However, individual features of each of these polymers also make a contribution to the observed regularities.

For instance, in the case of highly crystalline starch polysaccharides, the primary cryogel is formed immediately during freezing, while thawing conditions influence the further formation (adjustment) of intermolecular contacts.[214, 235] This can be clearly seen in a study of dilute systems in which the freezing–thawing cycle affords a cryoprecipitate rather than a cryogel over the whole specimen bulk. The precipitate can be readily separated from the solution and the yield of the gel fraction can be measured. This parameter shows that a decrease in the rate of thawing results in a substantial increase in the structurisation efficiency compared to that in rapidly thawed preparations.[235]

To conclude this Section, it should be noted that non-covalent intermolecular interactions play an important role also in the formation of cryogels based on colloidal dispersion systems: suspensions, pastes and colloid sols. Such cryostructurates are often produced on freezing–thawing of dispersions of food biopolymers; they are formed as coagulates upon cryogenic treatment of latexes, and so on. However, subtle physicochemical mechanisms of these processes have not yet been adequately studied, although the need for these studies is obvious.

Generally, the formation of non-covalent (physical) polymeric cryogels is characterised by the same effects as covalent cryostructuring, *i.e.*, the formation of macroporous gel-like bodies, apparent decrease in the CCG, acceleration of gelation over a definite range of negative temperatures and extremal temperature dependences of process rates. The main difference between non-covalent cryotropic gelation and the processes of formation of covalently cross-linked cryogels is the important role of the thawing stage (incubation at subzero temperatures) in the former case.

V. Applications of cryotropic gelation processes and materials based on polymeric cryogels

In recent years, the interest in polymeric cryogels as not only objects of scientific research but also promising materials for the solution of applied problems has constantly increased. The range of applications of the cryotropic gelation techniques and the gel materials is fairly broad. The unique porous structure of cryogels proves to be useful in many cases. An especially large number of publications including reviews [3, 4, 6, 10, 11, 269] have been devoted to the use of various cryogels as materials for biomedical and biotechnological purposes. The development of cryogenic processes for the production of new food forms, new sorbents, filters, mechanochemical gel actuators (manipulator mechanisms), leather-like materials, catalyst systems, and many other things is in progress. The data demonstrating the fields and branches in which diverse cryogels have already been successively used are summarised in Table 5. It should be emphasised that the range of

Table 5. Fields of application of cryotropic gelation processes and materials based on polymeric cryogels.

Gel-formimg reagents	Cryogel type	Application of a material or cryotropic gelation procedure	Ref.
2-HOEMA	IV.1(*r*)	carriers for enzyme immobilisation	3, 140, 142, 149 – 153, 158, 160 – 164
2-HOEMA + acrolein	IV.1(*r*)	carriers for antibody immobilisation and preparation of immunosorbents	143, 172
2-HOEMA + gialuronic acid	IV.1(*r*)	gel matrices for controlled drug release	165
Hydroxy- and methoxyoligoglycol methacrylates	IV.1(*r*)	carriers for immobilisation of microbial cells	141, 144 – 148, 156, 157, 159, 166
Oligoethylene glycol monometh-acrylates and dimethacrylates	IV.1(*r*)	gel matrices for cultivation of animal cells	168
AAM + MBAAM	IV.1(*c*)	carriers for immobilisation of microbial cells	22, 270, 271
	IV.1(*c*)	gel bases for supermacroporous bioaffinity sorbents	269, 272
AAM + MBAAM + allyl glycidyl ether	IV.1(*c*)	gel bases for supermacroporous bioaffinity sorbents	269, 272
Cryo(silica gel)	IV.1(*pc*)	carriers for immobilisation of microbial cells	22, 273
Gelatin + formaldehyde	IV.2	immobilisation of yeast cells	274
Serum albumin + glutaraldehyde	IV.2	immobilisation of photosynthetically active cell organelles of lettuce	275 – 278
	IV.2	immobilisation of the submembrane fraction of photosystem II of spinach cells	279, 280
	IV.2	immobilisation of photosynthetically active cyanobacteria	281
	IV.2	immobilisation of thermophilic bacterial cells	282
Polystyrene – polybutadiene block copolymer + Pd or Rh salts	IV.2	preparation of heterogeneous catalysts containing zerovalent metal nanoparticles	283
Collagen + dialdehyde (diepoxide, epichlorohydrin, diisocyanate, *etc.*)	IV.2	collagen materials for medical purposes	22, 284 – 287
A dispersion tanned by heavy metal salts of leather-processing wastes in the presence of cross-linking agents	IV.2	leather-like materials for domestic and technical purposes	22, 288 – 290
Sodium alginate + calcium gluconate	IV.3	polymeric scaffold of implants containing adsorbed hepatocytes	291, 292
Sodium alginate + calcium salt with a negative temperature factor of solubility	IV.3(*c*)	alginate sponges for medical purposes	22, 208, 209
Chitosan + β-tricalcium phosphate	IV.3(*c*)	polymer matrix of bone tissue implants	293
Polymeric acids	IV.3(*d*)	highly porous adsorbents for preconcentration of volatile bases	210, 294
Polymeric bases	IV.3(*d*)	highly porous adsorbents for preconcentration of volatile acids	211
PVA	IV.4	gel materials for calibration of NMR tomographs	266, 295, 296
	IV.4	immobilisation of AOT micelles containing enzymes	262
	IV.4	preparation of composites containing supported catalyst particles as fillers	297 – 299
	IV.4	gel matrices for controlled drug release	11, 300 – 306
	IV.4	carriers for enzyme immobilisation	307 – 325
	IV.4	carriers for immobilisation of microbial cells	6, 10, 22, 224, 252, 326 – 381
	IV.4	carriers for immobilisation of animal cells	382
	IV.4	gel bases for solid nutritional media for microbe cultivation	6, 22, 383
	IV.4	gel bases for solid nutritional media for plant cell cultivation	6, 22, 384
	IV.4	Support for the preparation of immunosorbents meant for isolation, purification, and modification of viruses	385, 386
	IV.4	chromatographic materials	387
	IV.4	protective coatings for frozen fish and meat	388 – 392
	IV.4	composite ice as a structural material	22, 393
	IV.4	thawed soil densification	22, 394
	IV.4	artificial baits for sports and amateur fishing	395
	IV.4	biocompatible materials for medical purposes	396 – 405
	IV.4	macroporous membranes	406, 407
PVA + chitosan or dextran	IV.4	biocompatible gel materials	408, 409
PVA + polyacrylic acid	IV.4	gel matrix for controlled drug release	168, 261, 410
PVA + alginate	IV.4	support for immobilised microbial cells	411 – 413
PVA + polyelectrolyte	IV.4	gel materials for mechanochemical actuators	7, 414 – 422
PVA + gellan	IV.4	gel matrix for controlled drug release	423
PVA + collagen fibres	IV.4	biocompatible gel materials	248, 424, 425
PVA + Fe salts	IV.4	gel dosimetric system	426
Solutions and dispersions of food biopolymers	IV.4	production of structured food forms	236 – 243, 427 – 433

problems solved using cryogels is permanently extending. In Table 5, types of cryogels are designated according to the numbers of the Sections of the review in which these cryogels are considered:

IV.1 are cryogels formed upon polymerisation (*r* implies radiation initiation, *c* means chemical initiation) or polycondensation (*pc*);

IV.2 are cryogels prepared by covalent cross-linking of polymeric precursors;

IV.3 are ionic cryogels (the letters correspond to the pathways of cryogel formation presented in Fig. 12);

IV.4 are non-covalent cryogels with a physical network of the polymeric phase.

VI. Conclusion

To summarise the discussion on the most important features of cryotropic gelation of polymeric systems, the properties of the gel specimens thus formed and some applications of these materials, several conclusions can be drawn.

1. Cryotropic gelation and concomitant chemical reactions (including the non-covalent interactions between the precursor molecules) are liquid-phase processes occurring in the non-frozen liquid microphase of moderately frozen (macroscopically solid) specimens.

2. Cryotropic gelation can take place in both aqueous and organic frozen media provided that the solvent used and the conditions of cooling ensure crystallisation rather than vitrification of the system.

3. The final products of cryotropic gelation (cryostructuring) are macroporous, often spongy gel-like materials called cryogels (cryostructurates). The role of the porogen in the formation of these polymeric materials is played by crystals of the frozen solvent; therefore, the pattern of porosity of the cryogels is determined by conditions of freezing, incubation in the frozen state, and, in some cases, thawing of the gelling system. The polymeric network of the gel phase (walls of macropores) possess intrinsic microporosity.

4. Due to the accumulation of soluble components in the non-frozen liquid microphase, cryotropic gelation is characterised by an apparent decrease in the CCG with respect to that observed for the gelation in the same initial systems at positive temperatures.

5. As a rule, moderate freezing of the initial system accelerates gelation.

6. The temperature dependences of the rate and efficiency of cryotropic gelation usually pass through an extremum due to the competition between factors that favour (cryoconcentration effects) and hamper (low temperature, high viscosity, and so on) the process.

7. The properties of the polymeric cryogels are determined by the temperature of the cryogenic process, the nature of the solvent used, the type (monomer or polymer precursors) and the concentration of gel-forming reagents, the presence of other soluble materials and insoluble fillers, the rate of freezing, the duration of storage in the frozen state, thawing dynamics, and some other factors.

8. A unique combination of a number of properties, first of all, high porosity and interconnection of macropores and the facility of the process technology for the preparation of gel materials with this type of morphology allow cryogels to be regarded as a new type of polymer systems interesting in both scientific and applied aspects. The cryotropic gelation technique opens up prospects for the perfection of existing materials and development of new materials for various practical applications.

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