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Synthesis and physicochemical properties of macroporous cryogels

The current review reveals the analysis of recent research in the field of synthesis and characterization of cryogels. The theoretical aspects of the process of cryotropic gelation are considered. The influence of freezing temperature and concentrations of initial monomer mixture on the pore size of the material is discussed. The concept of process of cryotropic gelation is revealed. The potential need for further research in the field of cryotropic gelation is highlighted.
Key words: hydrogels, cryotropic gelation, cryogels, cryopolymerization

Introduction

Hydrogels are defined as a cross-linked three-dimensional polymeric networks, insoluble in water and hydrophilic media. Hydrogels exhibit the unique ability to absorb large amounts of water or other biological liquids [12]. Hydrogels attracted a great interest of researchers and significant progress was achieved both in their synthesis and applications biomedical, biotechnological and pharmaceutical areas. Polymeric hydrogels can be used as water supersorbent materials [7], contact lenses [7], wound dressings [14 , 35], drug delivery [31, 21, 36, 37].

Stimuli-responsive nano- and microporous gels may be applied as drug delivery systems, biological sensors and materials for separation and purification of cells, organelles and proteins. Amphoteric microgels attracted a great interest due to their ability to a reversible phase transitions upon changing external conditions (e.g. pH, temperature and ionic strength of solution). Amphoteric microgels contain acidic and basic functional groups, which provide macromolecules positive and negative charges, as well as a isoelectric point. Depending on pH of the solution amphoteric gels may exhibit polyelectrolyte properties. For example, a recent study showed the possibility of polyelectrolite complex formation under cryo-conditions, which resulted in macroporous amphoteric hydrogels [14 , 25]. For polyelectrolytes these interactions occur simultaneously and compete against each other. The response of amphoteric nano- and microgels to the changes in external environment typically takes place within seconds.

Most studies were so far focused on the application of amphoteric nano- and microgels as stimuli-responsive systems [22 , 29 , 32, 38-46]. In most cases, the properties of these amphoteric particles can be changed under an external stimuli, which finally reflects on the size, structure and nature of interactions. The volume phase transitions occur in polymer gels as a result of many factors such as competing interactions, elasticity of polymer chains, osmotic pressure, H-bonding, hydrophobic and van der Waals interactions [2 , 25 , 26].

Among polymer hydrogels macro- and super-porous polymeric cryogels attracted a particular interest. These hydrogels are widely used in biotechnology [18] and regenerative medicine as

- effective carriers of immobilized enzymes and live cells; [5 , 13 , 19 , 35].
- matrices of immunosorbents and affinity sorbents for use with biological nano- and microparticles; macroporous scaffold for various metal nanoparticles [9].
- substrates for cultivation of animal cells;
- special matrices composed of mainly whole bacterial cells [1 , 15].

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Macroporous structure of cryogels provides unhindered diffusion of substrates to the immobilized active principles and the withdrawal of products, when cryogels used as a carrier of immobilized biocatalysts. Supermacroporous spongy cryogel structure can be effectively used for chromatographic separation and purification of biological macromolecules and live cells [8 , 10 , 20 , 23].

Polymeric cryogels as an object of research

Cryogel is a macroporous hydrogel in which ice crystals were used as a porogen during gelation process. The last two decades revealed a growing interest towards polymeric cryogels as an object of fundamental research and as a promising material for application in various areas (Fig. 1).

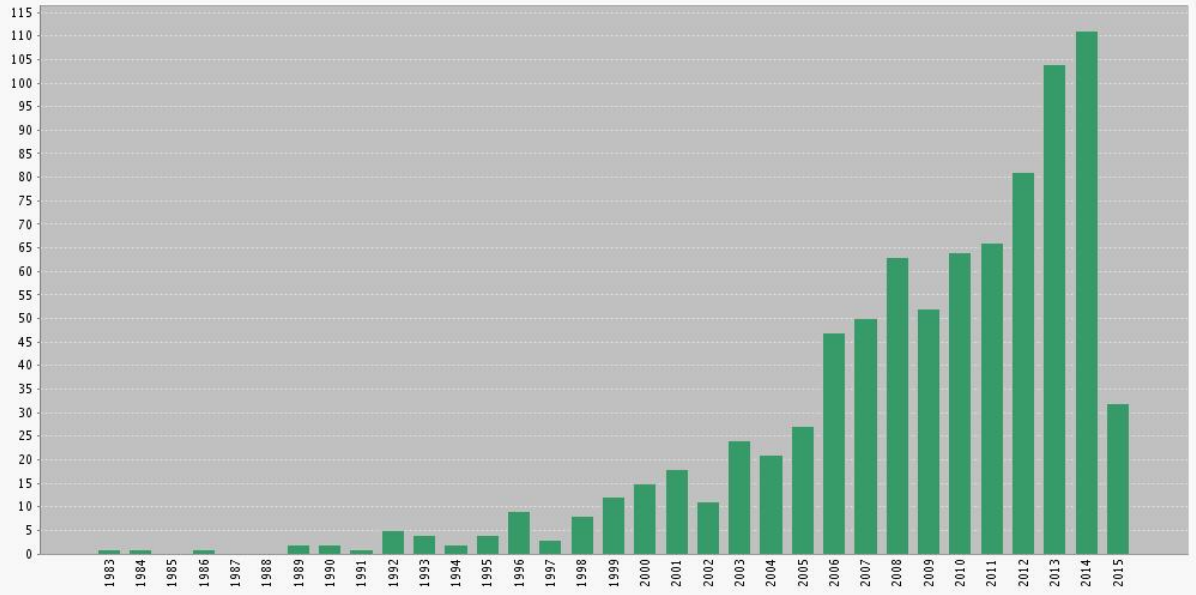

Figure 1 – The growth in the publications on «cryogels» as revealed by the analysis of ISI Web of Science

The areas of application of macro-porous materials is continuously expanding. New cryogenic technologies have been developed for food industry. Filters, sorbents [4], mechanochemical gel actuators, leather-like materials [33], catalytic systems are currently being developed [9]. Macroporous polymeric materials are commonly used in biotechnology and biomedicine [13, 24]. There are many different approaches for macroporous materials preparation such as lyophilization, cavitation, microemulsion polymerization, gas bubbling, and phase separation [11].

Polymeric cryogels are macroporous heterophase systems in which formation of pores occurs through a crystallization of a solvent. The specific feature of cryogels is their macroporous structure. The growth of each ice crystal is continuing until close contact with other growing crystals where all solutes are expelling into a non-frozen liquid microphase. Thus, the radical polymerization or cross-linking of polymers (natural, synthetic, bio molecules etc.) is taking place in a liquid microphase. After reaction occurred the thawing of the system leads to melting of ice crystals where the polymer network remains unaffected, which repeats the geometrical form of ice crystals, therefore the material has interconnected pores structure [5 , 16 , 18 , 28].

It worth to note that cryogels can also be prepared from low molecular weight compound without the use of any cross-linking agents or macromolecules and even without formation of chemical bonds.

Specific features of cryogelation process at subzero temperatures

Effects which are used in the synthesis of organic materials in aqueous solutions were named cryostructuring and cryogelation. These materials were named «cryogels» (from Greek κριοσ (cryo) – ice) [5, 16, 18 , 28, 47]. Some of the first reports of using the cryo-structuring phenomena for the preparation of supermacroporous hydrogels were published by the group of Lozinsky in the 1980s [28].

Thus, cryogelation is the formation of polymers, proteins or composites at temperatures below the freezing point of a solvent [19, 23]. It starts from freezing of an initial reaction mixture, by keeping it frozen for certain period of time to allow for the pore formation with a precursor cryo-concentrated in residual liquid microlayers, influenced by microcrystallites of frozen bulk solvent, with physical or chemical cross-linking of the compounds forming macropore walls and following thawing at room temperature.

By varying the characteristics of the polymers used for cryogel synthesis (molecular weight, molecular weight distribution, polymer concentration in the system, solvent composition etc.), as well as processing conditions of cryogelation (temperature, freezing time, freezing rate, number of thawing cycles etc.) it is possible to regulate a wide range of physicochemical parameters, micro- and macrostructure of the final gels [16].

Some special effects are observed when the freezing of the reaction mixture occurs under parameters far out of balance, for instance, dependence of cooling rate of the sample on polymerization rate; reaction vessel geometry and the chemical nature of reaction vessel walls; holding time in a frozen state; the amount of water in the reaction mixture and many other factors [5 , 12].

From a thermodynamical point of view, the cause of formation of liquid microphase in a multicomponent frozen solutions is that the incorporation of the solutes into a solid lattice of the solvent requires more energy than is expended to raise the chemical potential with increasing concentrations of the components in the liquid microphase [16].

Typically, when the freezing rate is high, then fine polycrystals of freezable liquid are formed. The size of ice crystals for aqueous solution of polyvinyl alcohol (PVA) (7%) frozen at -10^0C is several times lower than that for 14% solution of PVA- and last one by 1-2 orders lower than the ice crystals obtained from pure water[5, 48].

From practical point of view of characterization of cryogels there are some important characteristics such as specific surface area(S), pore volume (Vp), pore size distributions (PSD), pore wall thickness distribution (PWTD), pore connectivity and tortuosity. Typically, cryogels are micro/macroporous materials (average pore diameter in the range of $1<d<300$ μm) with pore walls of several micrometers in thickness. According to the life sciences classification, pore sizes at diameter $d_{nano}<0.1$ μm, $0.1<d_{micro}<100$ μm, and $d_{macro}>100$ μm correspond to nano-, micro-, and macropores, respectively [12]. The formation of cryogels with internal nanoporosity is a complicated task. Recently, the possibility of cryogel formation with additional internal nanoporosity in the pore walls was shown [14]. Ozmen et. all showed the dependence of microporosity of polyacrylamide cryogel from temperature and composition of solvent mixture [17].

There are some difficulties in quantitative characterization of the native texture of cryogels because of their softness and highly hydrated structure. Therefore, in most published studies the structural and textural characteristics of cryogels were carried out only on a qualitative or semi-quantitative level [14, 17, 25, 49]. Representative examples of these characteristics are the microscopic images of dried or freeze-dried cryogels without determination of the above mentioned parameters (Vp, S, PSD, and PWTD) or with an estimation of average pore size and porosity [3]. However, in some studies, PSD and other textural characteristics were determined in detail by using mercury porosimetry and image analysis [4, 27, 50]. Currently our group is working under development and investigation of amphoteric cryogels composed of various compositions of dimethylaminoetylmethacrylate (DMAEMA) and methacrylic acid. As shown in Figure 2 the pore size of the cryogel can be roughly estimated by the use of Image J program. Cryogels DMAEMA-MAA characterized by well distributed microporosity with mean diameter of pores 75 μm (with 2,5% MBAA as a crosslinking agent) and 44.7 μm (with 2,5% MBAA as a crosslinking agent).

Using high magnification SEM image of the cryogel one can graphically estimate average thickness of walls.

The variation of the freezing temperature during cryopolymerization process allows to regulate the morphology of macroporous materials. This approach was used to obtain cryogels based on the so-called «stimuli-responsive» or «sensitive» polymers macroporous hydrogels [23]. These cryogels have the ability of a reversible collapse at change of parameters of the environment, are considered as promising materials for biomedical and biotechnological applications.

Thermosensitive cryogels, where a chain conformation of an appropriate linear polymer is reversibly changing when passing through the upper or lower critical solution temperature (LCST or UCST), capable of significant thermo-induced reversible changes in the degree of swelling. These systems include cryogels based on pNIPAAm or poly-N-vinylcaprolactam [5].

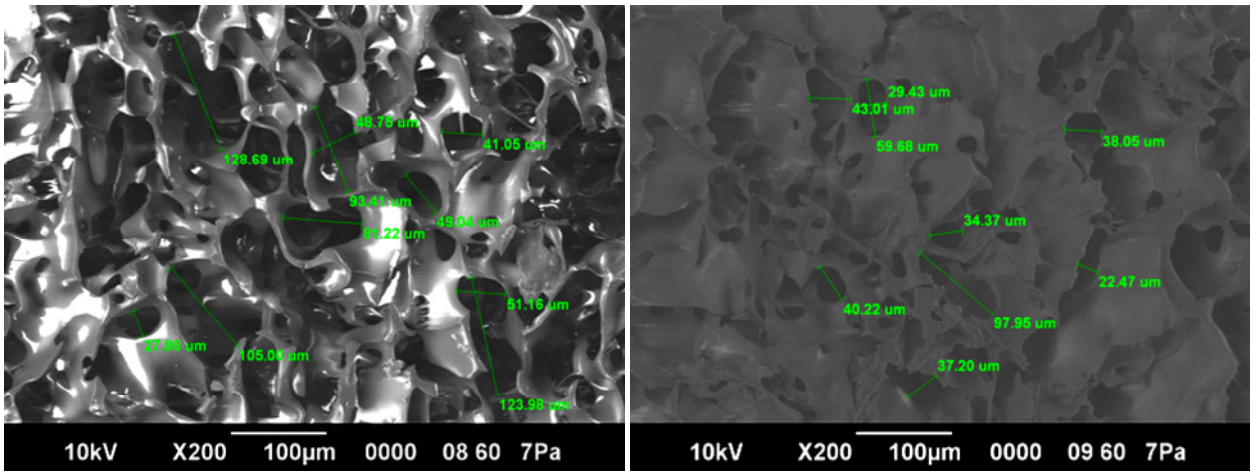

Figure 2 – SEM images of DMAEMA-MAA 1:1 with 2,5% MBAA (left), with 10% MBAA (right)

Thus, pNIPAAm cryogels rapidly and reversibly react to minor changes in the environment can be called «smart» cryogels [30 , 34]. It is known that under heat treatment of aqueous solution of pNIPAAm the hydrophobic aggregation is taking place at 32^0C, which leads to phase separation in the system [26]. The degree of swelling of pNIPAAm cryogel determined at sub-zero temperatures was much smaller than that for the pNIPAAm hydrogel synthesized at 22^0C [5].

This phenomenon can be explained by the swelling of pNIPAAm cryogel depending on concentration of monomers in the initial monomer mixture and the degree of cross-linking. The study of cryogel containing pNIPAAm at temperature range from 4 to 40^0C revealed a sharp rapid phase transition at 30^0C. As shown in Figure 3, the thermosensitivity of pNIPAAm cryogel is a reversible process, where a rapid response of the gel to change of temperature takes place in oscillatory regime at 19^0C and 37^0C. The lower the concentration of monomers and the degree of crosslinking in pNIPAAm cryogel, the greater the changes in swelling and the greater the amplitude of the mechanical deformation occurring at temperature change from 19^0C to 37^0C [5].

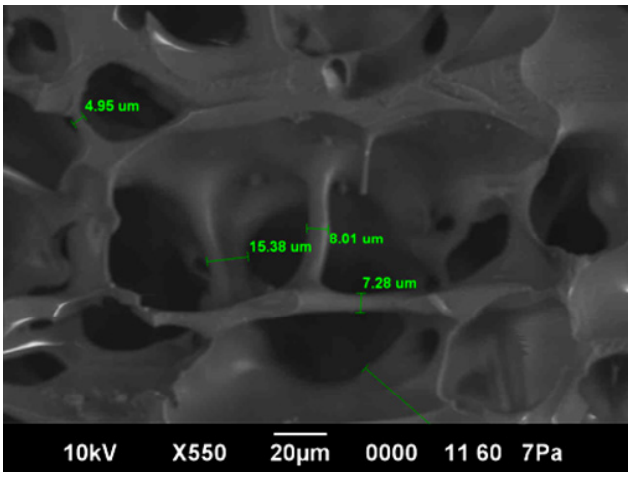

Figure 3 – SEM image of DMAEMA-MAA 1:1 with 5% MBAA as a crosslinking agent

The temperature and the rate of freezing

One of the most important parameters which controls the cryogelation process is the rate of freezing [28]. The pore size of cryogels depended on the rate of freezing in the following way: a low rate of freezing (or a higher temperature) the larger crystals of ice and therefore the larger pores in cryogels [28]. In order to avoid the surfusion the freezing temperature of reaction mixture should be sufficiently low, but not lower than the temperature of transition to glass state. The surfusion (or supercooling) is the process where the temperature passes the freezing point without formation of crystals of ice. This is the metastable state of water. The temperature of the formation of ice (or other solution) crystals related to the rate of freezing, volume of sample, nuclei forming agents. Variation of temperature during the freezing process allows to discern phase and structural changes in the system. Figure 5 shows the freezing curves of monomer mixture (acrylamide (AAm), methylene-bis-acrylamide (BisAAm), allylglycidyl ether) at temperatures -12, -20, -30^0C, respectively, where a phase states of the systems are well discerned. It is known that during the freezing of the reaction mixture at -12 °C the system exists in supercooled state, the following crys-

tallization process brings about the simultaneous abrupt rise of the temperature up to -11°C, indicating the crystallization of the solvent. After the reaction the mixture was transferred into cryostat with lower temperature the critical mass of nuclei of crystals formation reaches the limit within 2.6 minutes. The formation of nuclei of the crystals begins at -2.5°C. Cryoconcentration of solutes was reached through the freezing-out process of water from the solution. The increase in the viscosity of the unfrozen liquid phase slowed further crystallization, which completes in 8 minutes. The freezing of solutions at -20^{0}C or -30^{0}C in most cases completes with crystallization of solvent without the supercooling state. On the thermogram recorded at -30^{0}C a small peak appeared at 3.3 minutes related to the temperature -20^{0}C which is lower than the eutectic point for water/acrylamide system). This indicates the complete crystallization of liquid microphase, which exists in nonfrozen state at high temperatures [25].

4. Influence of concentration and composition of the initial monomer mixture

The increase in the concentration of monomers in the reaction mixture leads to an increase of polymer concentration in the non frozen phase, therefore formed pore walls have higher density and as a result the elastic modulus of the material is higher [14 , 35]. However this rule is right to some extent. Thus, the freezing of too concentrated monomer mixture brings about the formation of small ice crystals and as a consequence the pore sizes is small and mutual connection of macropores is low. This phenomenon is related to the smaller amount of the frozen solvent. As a result small ice crystallytes formed have poor connection with each other. For example, previously we had observed this phenomenon for amphoteric cryogel [4]. Amphoteric cryogel composed of equimolar composition of allylamine (AA) and methacrylic acid (MAA) (50/50 mol %) obtained from 10% initial monomeric mixture possessed a very low water permeability Figure 4 compare to the cryogel containing less ionic monomers in the initial monomer mixture. Thus, for cryogels composed of AAm-AA-MAA (80/10/10 mol %) and AA-MAA 50-50 mol% the difference in water permeability was about 60 times [4].

By adjusting the concentration and type of crosslinking agent cryogels with different pore sizes and pore wall thickness can be obtained.

Polyacrylamide cryogels (cryoPAAm) were synthesized by radical copolymerization of acrylamide (AAm) with N, N'-methylene bisacrylamide (BisAAm) in an aqueous solution. For initiation of radical copolymerization a redox system ammonium persulfate/ tetramethylethylenediamine (APS/ TEMED) was used, which generates radical ions even at low temperatures.

The critical concentration of gelation (CCG) is a concentration of monomer mixture, leading to formation of hydrogel. For instance, for preparation of polyacrylamide hydrogel at 20°C it is necessary to use 2% monomer solution composed of AAm and BisAAm. However, when the reaction mixture is frozen at -10 ºC immediately after initiating of radical polymerization it is possible to obtain a cryoPAAm using 1% of the same comonomers. The reduction of CCG in this case is an apparent decline, which is related to cryoconcentration effect. In fact the concentration of monomer in a non-frozen microphase where the polymerization is taking place is greater than that at room temperature. It is worth noting that this apparent decline of CCG is typical for all types of cryotropic gelation [5].

Cryogels based on acrylamide have a spongy morphology, which is mainly determined by temperature regimes of cryotropic gelation. Previously, the influence of freezing temperature on the morphology of the material has been shown. Thus, the structure of cryogels from solutions with constant concentration of the monomers prepared at -10 and -20°C and frozen in different ways. The monomer mixture solution placed in a cryostat with a predetermined temperature, where the freezing front was moving from above); an initial rapid freezing of mixture in liquid nitrogen to -196°C and the following transfer of samples to the cryostat at a predetermined moderate sub zero temperature. This technique minimizes the effect of the duration of cooling and freezing process on the kinetics of chemical reactions in so-called low-temperature hardening [5].

The formation of cryogel under this freezing regime results in decreasing of temperature from -10 to -20°C which leads to increase of the amount of porogen particles – ice crystals that significantly affects the diameter of the macropores in cryoPAAm [5]. Furthermore, the lower the temperature the more solvent is frozen, i.e. ULM volume becomes smaller, and the concentration of solutes therein becomes higher. Thus, the obtained pore walls are thinner, and built of more concentrated gel [13]. Using the technique of low-temperature hardening cryoPAAm morphology is changed. The architecture of the cryogel formed at –10°C combines the porosity of the sample obtained by above freezing regime, and the structure

of macropore walls of the material obtained at -20°C using above freezing regime. This means that during thawing of the frozen solution within a few minutes from -196 to -10°C the primary elements of the gel phase structure are forming. This is an evidence of rapid polymerization under cryoconditions. Additionally, the microporosity of cryogels can be regulated by introduction of special «molecular» porogen (e.g. oligoethyleneglycol with MW 1000 [6]), which after cryopolymerization can be washed out with excess of water. This leads to the formation of additional porosity of a certain size in the porewalls of cryogel. Another way to form an additional porosity inside of macroporous material is the use of foaming agents, which often leads to the formation of a certain amount of closed pores [27].

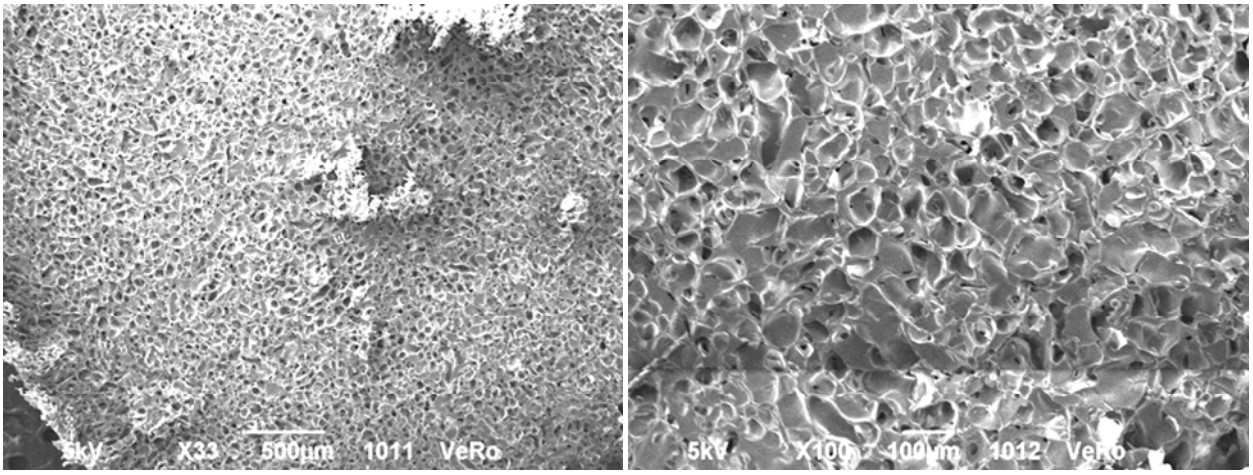

Figure 4 – SEM image of amphoteric cryogel AA-MAA 50-50 mol% at low x 33 (right) and high x 100 (left) magnifications.

Porous structure of various cryogels is a complex system composed of a 3D labyrinth, which is often observed in aqueous systems due to branched forms of polycrystalline ice, creating a complex configuration macropores in the bulk of a sample. The pore size and their shape can be changed though the directed crystallization of the solution, which allows to obtain cryogels with regularly oriented macropores. For example, the directional cooling of formamide solution brings about the crystallization of the solvent with formation of elongated crystals [2]. Using formamide and directed cooling process it is possible to prepare cryoPAAm regularly oriented macropores. In this case in the obtained cryogel contains a long capillary channels oriented in the direction of the temperature gradient [2]. Thus, it is possible within certain limits, to «manage» polymer cryogel macroporosity by adjusting the solvent and mode of its crystallization.

Osmotic characteristics (swelling in different media) of cryogel cryoPAAm mainly determined by factors such as the initial concentration of precursors[4], the nature of solvent and [17] the conditions of cryogenic treatment [5]. The total volume of liquid that absorbs cryogels consists of two components: solvate associated solvent and capillary solvent. From one side the solvate associated solvent is firmly connected with the polymer network, from the other side the capillary solvent fills the space in the macropores and can be simply removed through the gentle squeezing of cryogel structure [2].

The freezing and melting (thawing) process of frozen solutions of complex substances is a complicated process, which affected by various factors.

The process of freezing or thawing of the system solvent-polymer-low molecular weight additive is strongly influenced by the polydispersity of polymers. This leads to non-equivalence of phase diagrams of liquid-solid for the same pair of solvent – polymer with different molecular weights (MW) and molecular weight distribution (MWD) of high molecular weight component. Furthermore, a slow relaxation process in viscous non-frozen liquid phase in the frozen sample is strongly dependent on their thermal prehistory during freezing process.

Another problem, which makes the investigation of gelation process using a phase diagram more complicated, is self-association of polymers [9, 14, 28]. It is known that concentrated aqueous solution of polymers at low temperature have the ability to

spontaneous association. Therefore, in this case the obtained the phase diagram for the same system with the same sub zero temperature can be different. In each particular case the phase diagram is dependent on the dynamics of cooling of a sample and the rate of crystallization of a solvent [12].

Developing new simple and inexpensive methods of investigation of cryogelation process is a promissing direction for further research.

Thus, the physical and chemical properties of products of radical cryopolymerization can be widely changed through the variation of concentration and nature of monomer precursors. These scaffolds can be used as carriers for immobilization of enzymes, antibodies, protein-free biopolymers and different types of cells.

Conclusion

The cryotropic treatment of the reaction mixture does not stop the radical polymerization process. The cryogelation process is taking place in nonfrozen liquid microphase. The temperature and the regime of freezing, as well as composition of the monomer mixture determines the porosity of the material as the pore diameter, the thickness of walls, the area of internal surface. Two methods of cryogelation were discussed. The first one is the gradual cooling of the reaction mixture from room temperature to freezing point of the solvent and gradual thawing to ambient temperature. The second method is a rapid freezing of the solution in liquid nitrogen and the following gradual thawing of the sample to the temperature of crystallization of the solvent. The complete description of a porosity of cryogel structure can be performed via the determination of various parameters: pore volume (Vp), specific surface area (S), pore size distributions (PSD), pore wall thickness distribution (PWTD), then using these data and a modern software one can also calculate pore connectivity and tortuosity.

Acknowledgements

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References

1 Aragão Börner R., Zaushitsyna O., Berillo D., Scaccia N., Mattiasson B., Kirsebom H. Immobilization of Clostridium acetobutylicum DSM 792 as macroporous aggregates through cryogelation for butanol production // Process Biochemistry. – 2014. – 1//. – T. 49. – C. 10-18.

2 Berillo D., Elowsson L., Kirsebom H. Oxidized Dextran as Crosslinker for Chitosan Cryogel Scaffolds and Formation of Polyelectrolyte Complexes between Chitosan and Gelatin // Macromolecular Bioscience. – 2012. – Aug. – T. 12. – C. 1090-1099.

3 Berillo D., Mattiasson B., Galaev I. Y., Kirsebom H. Formation of macroporous self-assembled hydrogels through cryogelation of Fmoc-Phe-Phe // Journal of Colloid and Interface Science. – 2012. – 2/15/. – T. 368. – C. 226-230.

4 Berillo D., Volkova N. Preparation and physicochemical characteristics of cryogel based on gelatin and oxidised dextran // Journal of Materials Science. – 2014. – 2014/07/01. – T. 49. – C. 4855-4868.

5 Macroporous Polymers. / Bo M., Ashok K., IgorYu G.: CRC Press, 2009. – 1 c.

6 Chen J., Park K. Synthesis and characterization of superporous hydrogel composites // Journal of Controlled Release. – 2000. – Mar 1. – T. 65. – C. 73-82.

7 Cook M.T., Saratoon T., Tzortzis G., Edwards A., Charalampopoulos D., Khutoryanskiy V.V. CLSM Method for the Dynamic Observation of pH Change within Polymer Matrices for Oral Delivery // Biomacromolecules. – 2013. – 2013/02/11. – T. 14. – C. 387-393.

8 Dainiak M.B., Galaev I.Y., Mattiasson B. Macroporous monolithic hydrogels in a 96-mini-column plate format for cell surface-analysis and integrated binding/quantification of cells // Enzyme and Microbial Technology. – 2007. – 3/5/. – T. 40. – C. 688-695.

9 Dainiak M.B., Kumar A., Plieva F.M., Galaev I.Y., Mattiasson B. Integrated isolation of antibody fragments from microbial cell culture fluids using supermacroporous cryogels // Journal of Chromatography A. – 2004. – Aug. – T. 1045. – C. 93-98.

10 Dainiak M.B., Plieva F.M., Galaev I.Y., Hatti-Kaul R., Mattiasson B. Cell chromatography: Separation of different microbial cells using IMAC supermacroporous monolithic columns // Biotechnology Progress. – 2005. – Mar-Apr. – T. 21. – C. 644-649.

11 Fatima M.P., Igor Yu G., Bo M. Production and Properties of Cryogels by Radical Polymerization // Macroporous PolymersCRC Press, 2009. – C. 23-47.

12 Gun'ko V.M., Savina I.N., Mikhalovsky S.V. Cryogels: Morphological, structural and adsorption

characterisation // Advances in Colloid and Interface Science. – 2013. – 1//. – T. 187–188. – C. 1-46.

13 Hentze H.P., Antonietti M. Porous polymers and resins for biotechnological and biomedical applications // Reviews in Molecular Biotechnology. – 2002. – 3//. – T. 90. – C. 27-53.

14 Kirsebom H., Elowsson L., Berillo D., Cozzi S., Inci I., Piskin E., Galaev Igor Y., Mattiasson B. Enzyme-Catalyzed Crosslinking in a Partly Frozen State: A New Way to Produce Supermacroporous Protein Structures // Macromolecular Bioscience. – 2013. – T. 13, № 1. – C. 67-76.

15 Kudaibergenov S., Adilov Z., Berillo D., Tatykhanova G., Sadakbaeva Z., Abdullin K., Galaev I. Novel macroporous amphoteric gels: Preparation and characterization // Express Polymer Letters. – 2012. – May. – T. 6, № 5. – C. 346-353.

16 Lozinsky V.I. Cryogels on the basis of natural and synthetic polymers: preparation, properties and application // Russian Chemical Reviews. – 2002. – T. 71, № 6. – C. 489.

17 Lozinsky V.I. Polymeric cryogels as a new family of macroporous and supermacroporous materials for biotechnological purposes // Russian Chemical Bulletin. – 2008. – May. – T. 57, № 5. – C. 1015-1032.

18 Lozinsky V.I., Galaev I.Y., Plieva F.M., Savinal I.N., Jungvid H., Mattiasson B. Polymeric cryogels as promising materials of biotechnological interest // Trends in Biotechnology. – 2003. – Oct. – T. 21, № 10. – C. 445-451.

19 Lozinsky V.I., Plieva F.M., Galaev I.Y., Mattiasson B. The potential of polymeric cryogels in bioseparation // Bioseparation. – 2001. – T. 10. – №4-5. – C. 163-188.

20 Martynenko N.N., Gracheva I.M., Sarishvili N.G., Zubov A.L., El'Registan G.I., Lozinsky V.I. Immobilization of champagne yeasts by inclusion into cryogels of polyvinyl alcohol: Means of preventing cell release from the carrier matrix // Applied Biochemistry and Microbiology. – 2004. – Mar-Apr. – T. 40, № 2. – C. 158-164.

21 Morrison P. W. J., Connon C. J., Khutoryanskiy V.V. Cyclodextrin-Mediated Enhancement of Riboflavin Solubility and Corneal Permeability // Molecular Pharmaceutics. – 2013. – Feb. – T. 10. – №2. – C. 756-762.

22 Ogawa K., Nakayama A., Kokufuta E. Preparation and characterization of thermosensitive polyampholyte nanogels // Langmuir. – 2003. – Apr. – T. 19. Ц № 8. – C. 3178-3184.

23 Ozmen M.M., Dinu M.V., Okay O. Preparation of macroporous poly(acrylamide) hydrogels in DMSO/water mixture at subzero temperatures // Polymer Bulletin. – 2008. – Mar. – T. 60. – № 2-3. – C. 169-180.

24 Peppas N.A., Huang Y., Torres-Lugo M., Ward J.H., Zhang J. Physicochemical, foundations and structural design of hydrogels in medicine and biology // Annual Review of Biomedical Engineering. – 2000. – 2000. – T. 2. – C. 9-29.

25 Piskin K., Arca E., Piskin E. Radiopolymerized mixture of acrylic-acid, methyl-methacrylate, and polyethylene-glycol as an enzyme support system // Applied Biochemistry and Biotechnology. – 1984. – T. 10. – C. 73-79.

26 Plieva F., Xiao H. T., Galaev I. Y., Bergenstahl B., Mattiasson B. Macroporous elastic polyacrylamide gels prepared at subzero temperatures: control of porous structure // Journal of Materials Chemistry. – 2006. – T. 16. – № 41. – C. 4065-4073.

27 Plieva F.M., Karlsson M., Aguilar M.R., Gomez D., Mikhalovsky S., Galaev I. Y. Pore structure in supermacroporous polyacrylamide based cryogels // Soft Matter. – 2005. – Oct 14. – T. 1. – № 4. – C. 303-309.

28 Rogozhin S.V., Lozinskii V.I., Vainerman E.S., Korshak V.V. The influence of freezing of the polymerizing monomer solutions on the molecular-weight of polymers obtained // Doklady Akademii Nauk Sssr. – 1983. – 1983. – T. 273. – № 5. – C. 1140-1143.

29 Schachschal S., Balaceanu A., Melian C., Demco D.E., Eckert T., Richtering W., Pich A. Polyampholyte Microgels with Anionic Core and Cationic Shell // Macromolecules. – 2010. – May. – T. 43. – № 9. – C. 4331-4339.

30 Schild H.G. Poly (N-isopropylacrylamide) – experiment, theory and application // Progress in Polymer Science. – 1992. – 1992. – T. 17. – №2. – C. 163-249.

31 Storha A., Mun E. A., Khutoryanskiy V. V. Synthesis of thiolated and acrylated nanoparticles using thiol-ene click chemistry: towards novel mucoadhesive materials for drug delivery // Rsc Advances. – 2013. – T. 3, № 30. – C. 12275-12279.

32 Tan B.H., Ravi P., Tam K.C. Synthesis and characterization of novel pH-responsive polyampholyte microgels // Macromolecular Rapid Communications. – 2006. – Apr. – T. 27, № 7. – C. 522-528.

33 Tatykhanova G.S., Sadakbayeva Zhansaya K., Berillo D., Galaev I., Abdullin Khabib A., Adilov Z., Kudaibergenov Sarkyt E. Metal Complexes of Amphoteric Cryogels Based on Allylamine and Methacrylic Acid // Macromolecular Symposia. – 2012. – T. 317-318, № 1. – C. 18–27.

34 Yu H., Grainger D.W. Thermosensitive swelling behavior in cross-linked n-isopropylacrylamide networks - cationic, anionic, and ampholytic hydrogels // Journal of Applied Polymer Science. – 1993. – Sep 5. – T. 49, № 9. – C. 1553-1563.

35 Zaushitsyna O., Berillo D., Kirsebom H., Mattiasson B. Cryostructured and Crosslinked Viable Cells Forming Monoliths Suitable for Bioreactor Applications // Topics in Catalysis. – 2014. – Mar. – T. 57, № 5. – C. 339-348.

36 Zhao Q., Sun J.Z., Ling Q.C., Zhou Q.Y. Synthesis of Macroporous Thermosensitive Hydrogels: A Novel Method of Controlling Pore Size // Langmuir. – 2009. – Mar. – T. 25, № 5. – C. 3249-3254.

37 Lozinsky V.I. A Brief History of Polymeric Cryogels // Polymeric Cryogels: Macroporous Gels with Remarkable Properties. – 2014. – T. 263. – C. 1-48.

38 Dogan T., Bayram E., Uzun L., Senel S., Denizli A. Trametes versicolor laccase immobilized poly(glycidyl methacrylate) based cryogels for phenol degradation from aqueous media // Journal of Applied Polymer Science. – 2015. – T. 132, № 20. – C. 9.

39 Gorgieva S., Kokol V. Processing of gelatin-based cryogels with improved thermomechanical resistance, pore size gradient, and high potential for sustainable protein drug release // Journal of Biomedical Materials Research Part A. – 2015. – T. 103, №3. – C. 1119-1130.

40 Jackson N., Verbrugghe P., Cuypers D., Adesanya K., Engel L., Glazer P., Dubruel P., Shacham-Diamand Y., Mendes E., Herijgers P., Stam F. A Cardiovascular Occlusion Method Based on the Use of a Smart Hydrogel // Ieee Transactions on Biomedical Engineering. – 2015. – T. 62, № 2. – C. 399-406.

41 Kuo C.Y., Chen C.H., Hsiao C.Y., Chen J.P. Incorporation of chitosan in biomimetic gelatin/chondroitin-6-sulfate/hyaluronan cryogel for cartilage tissue engineering // Carbohydrate Polymers. – 2015. – T. 117. – C. 722-730.

42 Papancea A., Patachia S., Dobritoiu R. Crystal violet dye sorption and transport in/through bio-based PVA cryogel membranes // Journal of Applied Polymer Science. – 2015. – T. 132, № 17. – C. 12.

43 Percin I., Khalaf R., Brand B., Morbidelli M., Gezici O. Strong cation-exchange chromatography of proteins on a sulfoalkylated monolithic cryogel // Journal of Chromatography A. – 2015. – T. 1386. – C. 13-21.

44 Sahiner N., Seven F. A facile synthesis route to improve the catalytic activity of inherently cationic and magnetic catalyst systems for hydrogen generation from sodium borohydride hydrolysis // Fuel Processing Technology. – 2015. – T. 132. – C. 1-8.

45 Sahiner N., Yildiz S., Al-Lohedan H. The resourcefulness of p(4-VP) cryogels as template for in situ nanoparticle preparation of various metals and their use in H-2 production, nitro compound reduction and dye degradation // Applied Catalysis B-Environmental. – 2015. – T. 166. – C. 145-154.

46 Sahiner N., Demirci S., Sahiner M., Yilmaz S., Al-Lohedan H. The use of superporous p(3-acrylamidopropyl)trimethyl ammonium chloride cryogels for removal of toxic arsenate anions // Journal of Environmental Management. – 2015. – T. 152. – C. 66-74.

47 Uygun M., Akduman B., Uygun D. A., Akgol S., Denizli A. Dye functionalized cryogel columns for reversible lysozyme adsorption // Journal of Biomaterials Science-Polymer Edition. – 2015. – T. 26, № 5. – C. 277-289.

48 Van Rie J., Declercq H., Van Hoorick J., Dierick M., Van Hoorebeke L., Cornelissen R., Thienpont H., Dubruel P., Van Vlierberghe S. Cryogel-PCL combination scaffolds for bone tissue repair // Journal of Materials Science-Materials in Medicine. – 2015. – T. 26, № 3. – C. 7.

49 Loo S.L., Krantz W.B., Fane A.G., Gao Y.B., Lim T.T., Hu X. Bactericidal Mechanisms Revealed for Rapid Water Disinfection by Superabsorbent Cryogels Decorated with Silver Nanoparticles // Environmental Science & Technology. – 2015. – T. 49, № 4. – C. 2310-2318.

50 Tabakli B., Topcu A. A., Doker S., Uzun L. Particle-Assisted Ion-Imprinted Cryogels for Selective Cd-II Ion Removal // Industrial & Engineering Chemistry Research. – 2015. – T. 54, № 6. – C. 1816-1823.International Journal of Biology and Chemistry 7, №1, 10 (2015)