Cryogels via UV Irradiation

Petar D. Petrov and Christo B. Tsvetanov

Contents

Abstract An effective and facile method for the synthesis of chemically crosslinked supermacroporous polymer cryogels based on UV irradiation is reviewed. The influence of key factors like the irradiation dose, temperature of freezing, concentration of polymer or monomer precursor, molar mass of polymer precursor, and the type and amount of the photoinitiator on crosslinking efficiency is discussed. The versatility of the method for preparation of a large number of biocompatible, biodegradable, and/or stimuli-responsive cryogels is demonstrated. Examples include some specific properties of well-investigated polyacrylamide (PAAm) and poly(*N*-isopropylacrylamide) (PNIPAAm) cryogels obtained by photocrosslinking as well as novel cryogels based on cellulose derivatives, hydrophobically modified polyglycidol (PGL), and ethoxytriethyleneglycol acrylate (ETEGA). Part of this review is focused on the applicability of supermacroporous cryogels as carriers of different species such as drugs, enzymes, nanoparticles, and cells immobilized in either cryogel walls (polymer matrix) or interconnected pores.

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Keywords UV irradiation • Photocrosslinking • Cellulose derivatives • Stimuli-responsive cryogels • Carriers

Abbreviations

AAm	Acrylamide
AgNPs	Silver nanoparticles
BBTMAC	(4-Benzoylbenzyl)trimethylammonium chloride
BisAAm	*N*,*N*′-methylenebisacrylamide
CNT	Carbon nanotube
DMAEMA	2-(Dimetylamino)ethyl methacrylate
DS	Degree of swelling
ETEGA	Ethoxytriethyleneglycol acrylate
GF	Gel fraction
HEC	2-Hydroxyethylcellulose
HEMA	2-Hydroxyethyl methacrylate
HPC	Hydroxypropylcellulose
HPMC	(Hydroxypropyl)methylcellulose
LCST	Lower critical solution temperature
MC	Methylcellulose
NIPAAm	*N*-Isopropylacrylamide
OEGMA	Oligo(ethyleneglycol) methacrylate
PAAm	Polyacrylamide
PEGDA	Poly(ethylene glycol) diacrylate
PEO	Poly(ethylene oxide)
PETEGA	Poly(ethoxytriethyleneglycol) acrylate
PGL	Polyglycidol
PHEMA	Poly(2-hydroxyethyl methacrylate)
PNIPAAm	Poly(*N*-isopropylacrylamide)
T_{VPT}	Temperature of volume phase transition
VCL	Vinyl caprolactam
UV	Ultraviolet

1 Introduction

Supermacroporous polymer cryogels are attractive materials due to their unique heterogeneous structure composed of large interconnected pores that are filled with solvent and surrounded by thin walls. Such a structure makes the diffusion of fluids and species within the volume of cryogel easy and, thereby, facilitates mass and heat transfer [1, 2]. Polymer cryogels are formed as a result of freezing of low or high molar mass precursors (dissolved most often in water), crosslinking, and subsequent thawing. A very important feature determining the success of

preparation of a heterogeneous open porous structure is that the solvent is frozen before the beginning of crosslinking reactions, i.e., the reaction system is cryostructured. In the case of chemical crosslinking by a redox system, which is one of the most frequently used approaches for synthesis of polymer cryogels, the polymerization rate has to be slow enough to prevent crosslinking of the system from the time of mixing of the reagents until the complete cryostructuration, i.e., any crosslinking in solution must be avoided [3]. Typically, when using a redox system, the time required for preparation of cryogels varies from 16 to 24 h [4–6].

From this point of view, the most efficient methods for regular cryostructuration of the system are those based on chemical crosslinking induced by high energy radiation (gamma-rays, electron beam) [7–9]. Here, the solution of reagents is first allowed to freeze, forming well-separated large interconnected ice crystals and nonfrozen liquid microphase. Then, the polymer network is formed. Usually, the irradiation procedure takes 60–180 min and saves time in the preparation of cryogels. However, the complex and expensive equipment needed in combination with the safety requirements seem to limit the wide use of these methods.

Ten years ago, the UV irradiation technique was successfully employed for the first time by our team for the synthesis of poly(ethylene oxide) (PEO) cryogels [10]. This method starts with freezing of the solvent and conducts the crosslinking reaction after the complete structuring of the system. Thus, together with full control over the formation of large-size crystals and nonfrozen liquid microphase, the method benefits from the facile procedure and easy access to a UV light source. By optimizing the experimental conditions, PEO cryogels of very high gel fraction (GF) yield (95 %) were obtained.

This review summarizes the recent achievements in preparation of various supermacroporous polymer cryogels via UV-induced crosslinking in partly frozen systems. The method is equally effective for the formation of cryogels from both water-soluble high molar mass linear polymers and vinyl monomers. Special attention is paid to some novel materials based on biodegradable and/or stimuli-responsive polymers and their application in some emerging fields, as well as the fabrication of nanocomposites with intriguing properties.

2 Cryogels from Water-Soluble High Molar Mass Polymers

Cryogels from high molar mass polymer precursors were obtained by a simple procedure involving preparation of semidilute/concentrated polymer solution (0.5–5 mass%) containing a photoinitiator, followed by freezing, UV-induced crosslinking, and thawing. Notably, the crosslinking reaction is very fast and formation of the polymer network can be completed within several minutes. The accepted mechanism of crosslinking of high molar mass linear polymers induced by UV light involves generation and subsequent recombination of macroradicals [10]

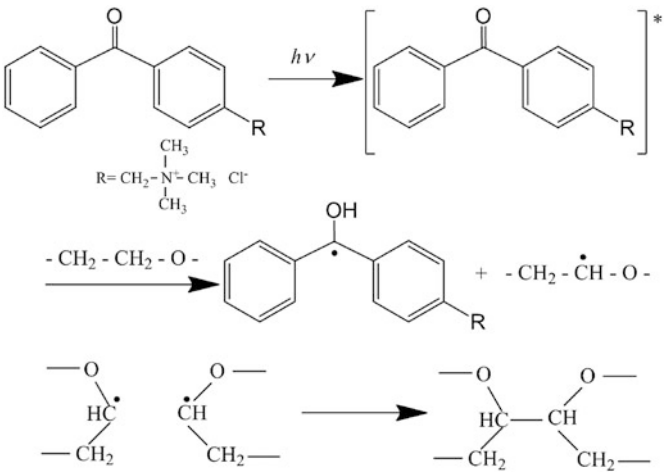

Fig. 1 Mechanism of photochemical crosslinking of PEO with photoinitiator (4-benzoylbenzyl) trimethylammonium chloride

with the aid of a photoinitiator. A substantial advantage of this method is the fact that no functional groups are required. Figure 1 shows one example of photocrosslinking of PEO in the presence of a water-soluble photoinitiator, (4-benzoylbenzyl)trimethylammonium chloride (BBTMAC). Upon UV irradiation, the benzophenone derivative undergoes several photophysical processes to afford an n,π^* triplet state, which then proceeds to reduction of BBTMAC and hydrogen atom abstraction from the PEO chain. Photocrosslinking occurs by a recombination reaction of two macroradicals to produce covalent bonds between two main chains.

One should point out that in the case of cryogels, the crosslinking reaction takes place in the nonfrozen liquid microphase, where the concentration of polymer is higher than in the initial solution due to the cryo-concentration phenomena. Therefore, under such conditions the probability for recombination of two macroradicals is increased. This fact explains why cellulose derivatives, which degrade in solution upon UV irradiation, were successfully crosslinked via cryogenic treatment [11, 12]. Figure 2 shows the storage (G') and loss (G'') moduli in the 0.1–10 Hz frequency range of 3 mass% aqueous 2-hydroxyethylcellulose (HEC) solutions irradiated with UV light in the frozen state (−30 °C) and at room temperature.

The two samples exhibit quite different rheological behavior. For the HEC solution irradiated at room temperature, a strong dependence of G'' on the frequency (f) and a cross-over at ca. 0.2 Hz were registered. Moreover, it was established that the viscosity of the initial solution was higher than that of the solution after irradiation at room temperature, which is evidence for the degradation of HEC macromolecules. In contrast, the apparent values of G' and G'' of the sample obtained from the frozen aqueous systems exhibited little dependence on f, and $G' > G''$ over the entire f range explored. The observed results are consistent with the typical behavior of polymer gels and indicate that crosslinking reactions

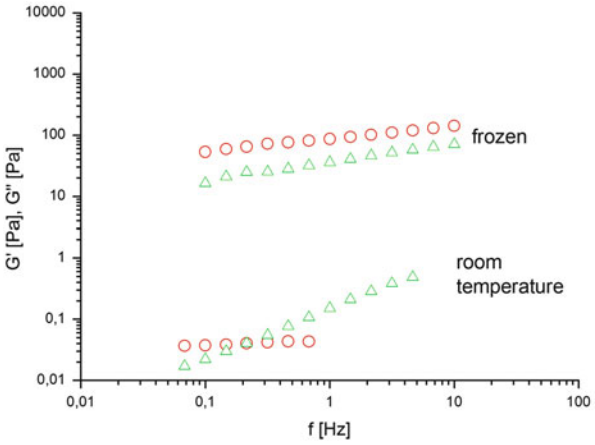

Fig. 2 Variation of storage G' (*circles*) and loss G'' (*triangles*) moduli in the 0.1–10 Hz frequency range of 3 mass% aqueous HEC (250,000 g/mol) solutions irradiated with UV–visible light at room temperature and in the frozen state (−30 °C); 5 mass% BBTMAC, irradiation time 2 min. Reprinted from [11] with permission from Elsevier

occur predominantly under the chosen experimental conditions. It has been described that two processes, degradation and crosslinking, are in competition at high-energy irradiation of cellulose derivatives with either an electron beam or gamma-rays [13–15]. When exposed to ionizing radiation at ambient temperature in the solid state and in aqueous solutions, the cellulose derivatives undergo degradation, whereas the best results for crosslinking have been obtained at paste-like conditions (25–40 mass%, depending on the polymer). Based on the theory of cryotropic gelation, it is assumed that most of the solvent forms crystals during freezing of aqueous solutions of cellulose derivatives, whereas the polymer, the photoinitiator, and the water molecules connected to the polymer through hydrogen bonds (non-freezable solvent) form a nonfrozen liquid microphase. Obviously, the polymer concentration in the liquid microphase is very high (cryoconcentration effect) and the reaction conditions closely resemble the conditions of the paste-like state. Therefore, during UV irradiation in the frozen state, the rate of crosslinking is much higher than the rate of chain scission reactions and a cryogel is formed.

Indeed, nonionic HEC, (hydroxypropyl)methylcellulose (HPMC), methylcellulose (MC), and cationic 2-hydroxyethylcellulose can be crosslinked via UV irradiation assisted by the cryogenic treatment (Table 1).

All cryogels based on cellulose derivatives are opaque materials and a significant part of the water (>65 %) can be separated easily by compression at low mechanical loads. A scanning electron microscopy (SEM) image of a HEC cryogel (Fig. 3) illustrates the typical supermacroporous structure of the material, which consists of large interconnected pores (50–200 μm) surrounded by thin walls.

The main factors affecting the efficiency of crosslinking and the properties of the material are the type and molar mass of polymer, the concentration of initial solution, the type and amount of photoinitiator, the temperature of freezing, and the irradiation dose. As a rule, each parameter has to be optimized to reach the maximum GF yield for given polymer, as exemplified below for HEC.

Table 1 Cryogels obtained via UV irradiation of frozen systems based on aqueous solutions of nonionic and cationic cellulose derivatives

Cellulose derivative	Molar mass (g/mol)	Gel fraction yield^{max} (%)	Degree of swelling
HEC[a]	1,300,000	95	13
HEC[a]	300,000	78	15
HEC[a]	90,000	51	22
Quaternized HEC[b]	900,000	75	12
HPMC[c]	120,000	50	22
MC[d]	88,000	46	25

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Experimental conditions: 2–5 mass% BBTMAC, irradiation time 2 min (irradiation dose 11.4 J/cm^2, input power 93 mW/cm^2, maximum wavelength at 365 nm)
HEC 2-hydroxyethylcellulose, *HPMC* (hydroxypropyl)methylcellulose, *MC* methylcellulose
[a]Degree of substitution (DS) 1.5; molar degree of substitution (MS) 2.5
[b]MS of quaternary ammonium moiety 0.4
[c]DS 1.1–1.6; MS 0.1–0.3
[d]DS 1.5–1.9

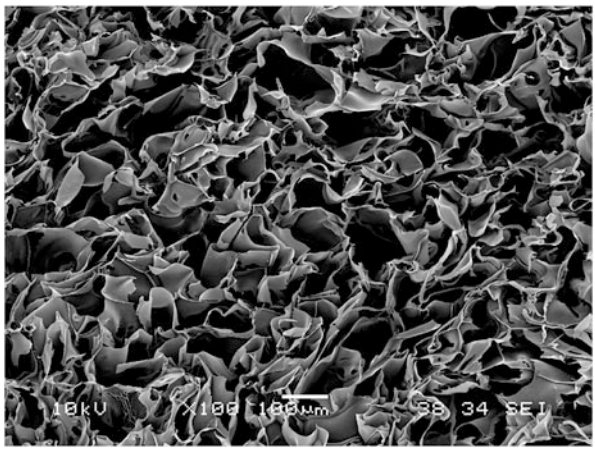


Fig. 3 SEM micrograph of HEC cryogel prepared at a freezing temperature of −20 °C, initial polymer concentration 1 mass%, polymer molar mass 1,300,000 g/mol, 2 mass% BBTMAC, irradiation time 2 min. Reprinted from [12] with permission from Elsevier

It was found that the GF yield of cryogels prepared by irradiation of frozen aqueous solutions of HEC (concentration 3 mass%, 5 mass% BBTMAC) with UV light at an irradiation dose rate of 5.7 J/cm^2 min increases with the irradiation time in the first 2 min and then reaches a constant value. Consequently, 2 min of irradiation is adequate for crosslinking of HEC in a frozen aqueous system and results in cryogels of good quality (the material maintains its integrity and original shape in water). The extremely short time required for the formation of a polymer network avoids the side effects of excessive heating during irradiation.

The dependence of the GF yield of HEC cryogels, crosslinked at −20 °C, on the initial concentration of HEC solutions is shown in Fig. 4.

Cryogels are formed at substantially low polymer concentrations, which is attributed to the cryo-concentration phenomenon. The GF yield increases with

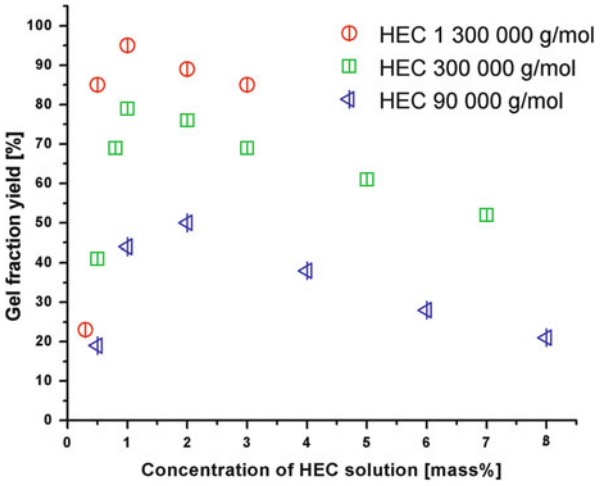


Fig. 4 Gel fraction yield of cryogels prepared from HECs of different molar masses at different polymer concentrations. Cryogels were obtained at a freezing temperature of −20 °C, 2 mass% BBTMAC, irradiation time 2 min. Reprinted from [12] with permission from Elsevier

increasing concentration and reaches a maximum at a polymer concentration of 1 mass% for HECs with molar masses of 1,300,000 g/mol and 300,000 g/mol, and 2 mass% for HEC with molar mass of 90,000 g/mol, and decreases at higher concentrations. In addition, the higher the molar mass of polymers, the higher the GF yield of cryogels.

HEC cryogels of good quality and high GF yield can be prepared by freezing the initial solution at a temperature between −15 and −30 °C at a cooling rate of 1 °C/min (Fig. 5). Note that the maximum value of GF yield is reached at −20 °C.

The use of an aromatic photoinitiator for preparation of HEC cryogels is not always desirable, especially when the materials obtained are intended for application in medicine and pharmacy. Therefore, studies have focused on the formation of cryogels of cellulose derivatives using hydrogen peroxide as a photoinitiator (Fig. 6) [12, 16]. H_2O_2 generates hydroxyl radicals during its photo-homolysis [17]. These radicals react with the polymer chains, giving rise to macroradicals that form a polymer network by recombination. The by-product of this reaction is water and, consequently, the material obtained can be used without additional purification. The GF yield of HEC cryogels obtained with H_2O_2 is slightly lower compared to BBTMAC at the same concentration (Fig. 6); however, one can prepare monolithic material that maintains its original shape and can be handled.

It is well known that cellulose derivatives are biodegradable polymers that undergo degradation by cleavage of the glycosidic linkages through the action of enzymes or microorganisms [15]. The process of enzymatic degradation of HEC cryogels has a specific feature. SEM analysis (Fig. 7) at an intermediate stage of degradation (12 h, 62 % weight loss) illustrates that the cryogel walls appear thinner and partially destroyed. This means that the enzyme molecules do not only attack the HEC network at the gel surface (typical for hydrogels), but that they also penetrate into the macroscopic pores and digest the whole polymer structure.

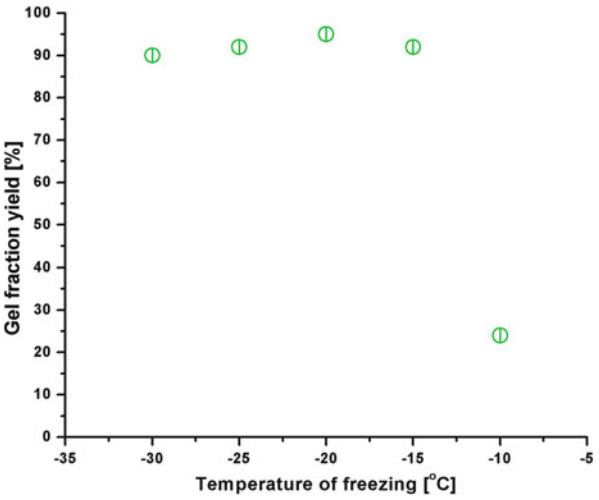


Fig. 5 Gel fraction yield of HEC cryogels obtained at various negative temperatures. Cryogels were prepared at initial polymer concentration 1 mass%, polymer molar mass 1,300,000 g/mol, 2 mass% BBTMAC, irradiation time 2 min. Reprinted from [12] with permission from Elsevier

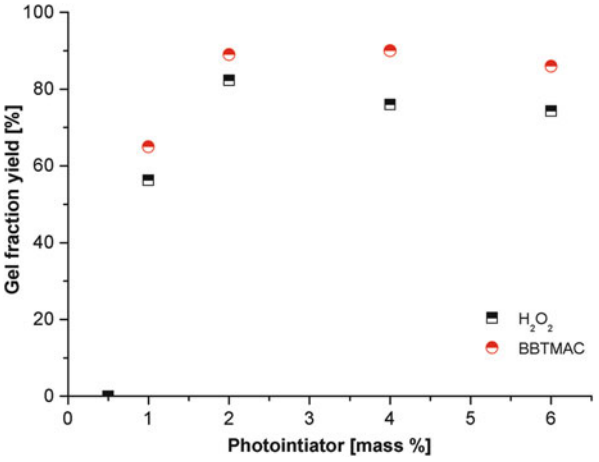


Fig. 6 Gel fraction yield of HEC cryogels prepared with different photoinitiators. Cryogels were prepared at a freezing temperature of −20 °C, initial polymer concentration 1 mass%, polymer molar mass 1,300,000 g/mol, 2 mass% initiator, irradiation time 2 min

On the other hand, it was found that HEC cryogels degrade slower than conventional HEC hydrogels of similar GF yield.

An appropriate strategy for increasing the GF yield and enhancing the mechanical strength of cryogels based on cellulose derivatives is to incorporate suitable crosslinking agents into the polymer network [11]. For instance, the storage modulus of HPMC cryogel prepared in the presence of 3 mass% *N*,*N*′-methylenebisacrylamide (BisAAm) is an order of magnitude higher than that of HPMC cryogel obtained without crosslinking agent. More interesting, the formation of a polymer co-network between HEC and another polymer such as chitosan (CS) leads to a material with significantly increased storage modulus and

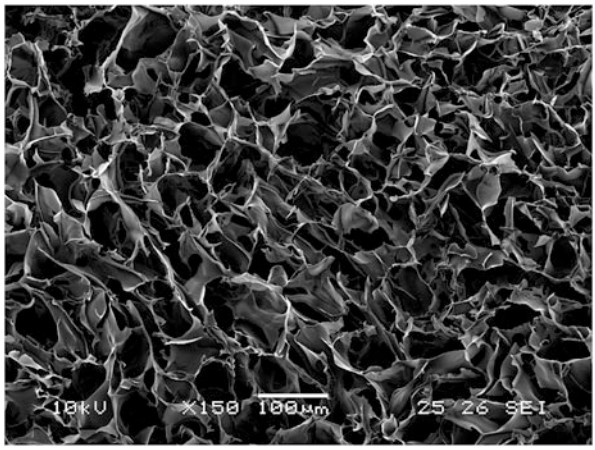


Fig. 7 SEM micrograph of HEC cryogel after 12 h action of the enzyme cellulase. Cryogel was prepared at a freezing temperature of −20 °C, initial polymer concentration 1 mass%, polymer molar mass 1,300,000 g/mol, 2 mass% BBTMAC, irradiation time 2 min. Reprinted from [12] with permission from Elsevier

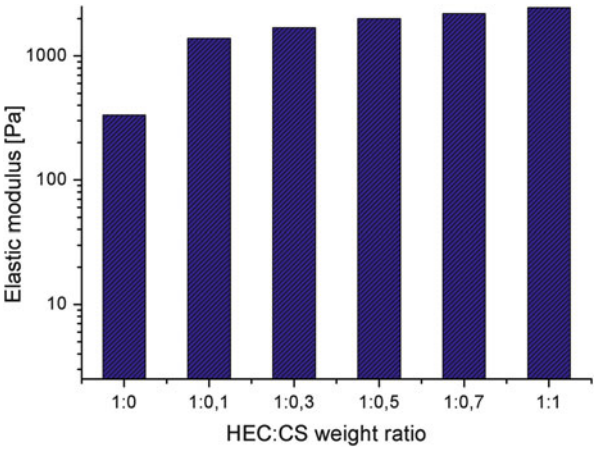


Fig. 8 Dependence of the elastic modulus G' of cryogels on the HEC-to-chitosan weight ratio. Cryogels were prepared at a freezing temperature of –20 °C, 5 mass% H_2O_2, 30 mass% BisAAm, initial polymer concentration 1.5 mass%, irradiation time 2 min. Reprinted from [18] with permission from Elsevier

pH-responsive properties [18]. The increase in chitosan content in the polymer co-network increases the mechanical strength of cryogels (Fig. 8).

Similarly, the degree of swelling (DS) of HEC–chitosan cryogels increases proportionally with increasing chitosan content. In acidic conditions, amino groups in chitosan are protonated and chitosan segments swell more at pH 4 than in neutral water. Thus, the DS of HEC–chitosan cryogels at pH 4 is higher than at pH 7 for all compositions studied (Fig. 9). The pH-triggered volume phase transition (swelling/shrinking) is a fast and reproducible process, i.e., HEC–chitosan cryogels can reach many times the defined DS in acidic or neutral water within 15–20 s.

The UV irradiation technique has also been successfully employed in the preparation of novel macroporous cryogels based on hydrophobically modified high molar mass polyglycidol (Fig. 10) [19]. It is known that by varying the degree of modification of polymer, it is possible to obtain temperature-responsive materials with tunable properties [20]. For all copolymer compositions studied

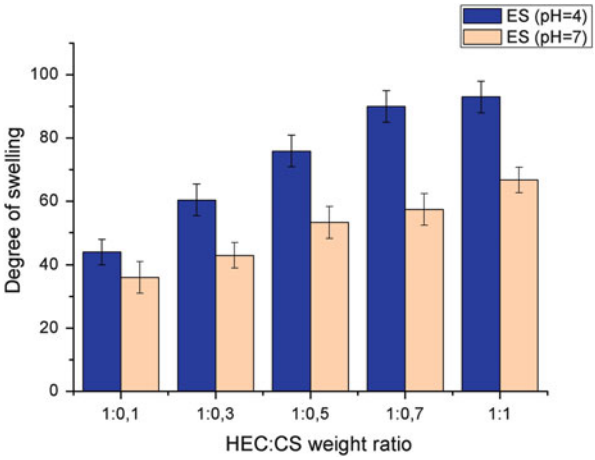


Fig. 9 Degree of swelling (*ES*) of HEC–chitosan cryogels of different composition in acidic and neutral water. Cryogels were prepared at a freezing temperature of −20 °C, initial polymer concentration 1.5 mass%, 30 mass% BisAAm, irradiation time 2 min. Reprinted from [18] with permission from Elsevier

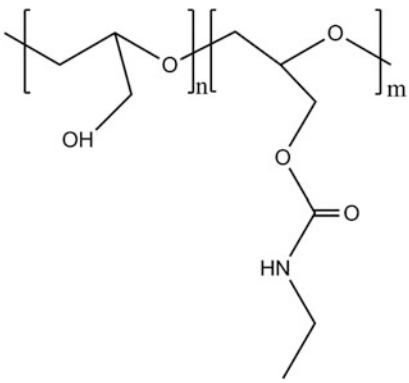


Fig. 10 Temperature-responsive poly(glycidol-*co*-ethyl glycidyl carbamate) (hydrophobically modified polyglycidol) precursor used for the synthesis of cryogels. Reprinted from [19] with permission from Elsevier

(copolymer molar mass 1,250,000 g/mol; molar degree of modification 32, 37, and 40 %), the maximum value of the GF yield was achieved at an initial copolymer concentration of 2 mass%, irradiation time 4 min (irradiation dose 22.8 J/cm^2), and 5 mass% BBTMAC.

Photochemical crosslinking of high molar mass polyacrylamide (PAAm) in a frozen aqueous system has been achieved as an alternative to the commonly used synthesis of PAAm cryogels from acrylamide. Advantageously, starting from a high molar mass precursor one may omit the use of crosslinking agent [16].

3 Cryogels from Water-Soluble Vinyl Monomers

It has been demonstrated by several teams that the UV irradiation technique can be used for the synthesis of polymer cryogels from different vinyl monomers (Table 2) in the presence of a photoinitiator and a crosslinking agent.

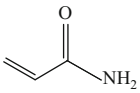
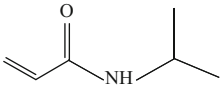
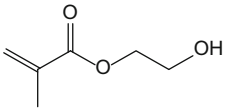
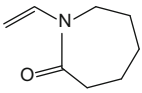
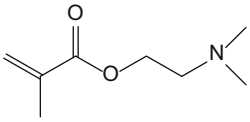
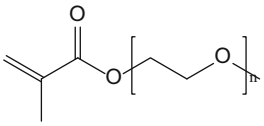
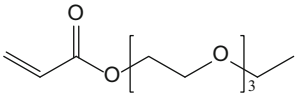
The polymer network is formed in the nonfrozen liquid microphase by a free radical photopolymerization/crosslinking reaction (Fig. 11) at defined negative temperature. The use of crosslinking agent provides the formation of a three-dimensional network instead of linear macrochains. Specifically, the concentration of reagents in the microphase is much higher than the concentration in the initial solution, due to the fact that a large amount of the solvent forms crystals.

The incorporation of crosslinks between the polymer chains also contributes to the mechanical strength of the material. A comparison between two polyacrylamide cryogels, synthesized from 5 mass% monomer solution containing crosslinking agent poly(ethylene glycol) diacrylate (PEGDA, 10 mass%) and 5 mass% polymer solutions without PEGDA, revealed that the latter cryogel has a storage modulus several times lower than the cryogel synthesized from monomer (Fig. 12). However, the addition of 10 mass% PEGDA to the polymer solution resulted in a cryogel with G' similar to that of AAm/PEGDA-based cryogels.

The choice of a powerful source of UV light seems to be the crucial factor for fast monomer conversion and formation of polymer network. The results from experiments carred out with a Dymax 5000-EC UV curing equipment with 400 W metal halide flood lamp emitting full spectrum UV–visible light at an irradiation dose rate of 5.7 J/cm^2 min (input power 93 mW/cm^2) showed that 5 min of irradiation is sufficient for preparation of disk-shaped cryogels of good quality [16, 21, 22]. Moreover, in the case of NIPAAm, ETEGA, and AAm, cryogels of extremely high GF yield (nearly quantitative monomer conversion) were obtained (Table 3). Thus, the as-synthesized materials do not contain undesirable monomer and crosslinking agent and can be directly used without any extraction procedure. The conversion of HEMA to a PHEMA network within the studied concentration range was also very high. Only vinyl caprolactam (VCL) cannot form cryogels of high GF yield, and the possible reason for the low crosslinking efficiency can be attributed to the poor solubility of VCL in water. The use of non-crystallizable co-solvent (ethanol; 10 vol%) seems to affect the regular cryostructuring and hinder the formation of polymer network.

In general, the time for preparation of polymer cryogels from vinyl monomers via UV irradiation is much shorter compared to the most often used technique, which is based on a redox system. Since the reagents can be dissolved quickly in the solvent and the reaction time is only 5 min, the freezing/cryostructuring of the system appears to be the longest stage in the preparation procedure. Usually, depending on the sample size and shape, the solvent can be frozen within several (up to 120) minutes, making completion of the entire process possible in less than 1 h. It should be noted that all the commercially available monomers listed in Table 3 can be used without any purification, which further facilitates the synthesis

Table 2 Monomer precursors used for the synthesis of polymer cryogels via the UV irradiation technique

Monomer	Structural formula	Solvent	References
Acrylamide (AAm)		Water	[16, 21]
N-Isopropylacrylamide (NIPAAm)		Water	[16]
2-Hydroxyethyl methacrylate (HEMA)		Water	[16]
N-Vinylcaprolactam (VCL)		Water/ ethanol	[16]
2-(Dimetylamino)ethyl methacrylate (DMAEMA)		1,4-Dioxane	[22]
Oligo(ethyleneglycol) methacrylate (OEGMA)		1,4-Dioxane	[22]
Ethoxytriethyleneglycol acrylate (ETEGA)		Water	[23, 24]

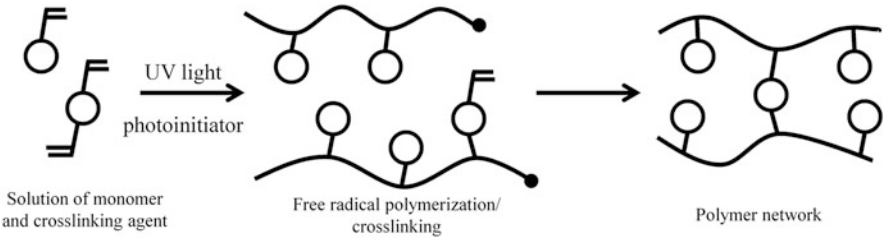


Fig. 11 Formation of polymer network from vinyl monomer and bifunctional crosslinking agent via UV irradiation

procedure. In addition, a very high crosslinking efficiency was reached without any purging of the reagent solution with an inert gas.

Certain limitations of the technique described in this review arise from the penetration depth of the irradiation. To fabricate material with identical behavior throughout the whole structure, it is recommended that the entire sample be

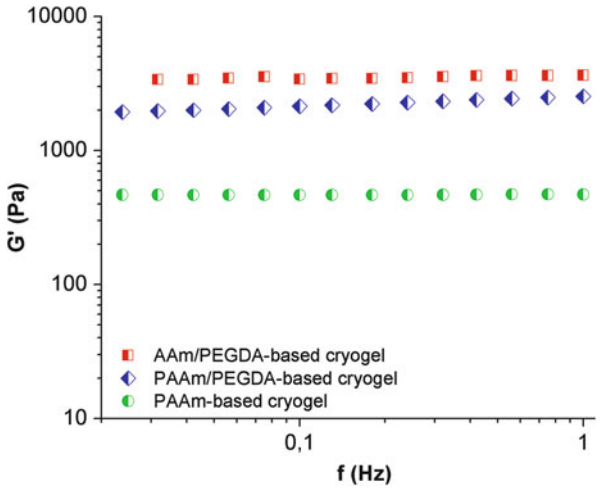


Fig. 12 Frequency dependence of the storage moduli G' of polyacrylamide cryogels prepared from 5 mass% monomeric (AAm) or polymeric precursors (PAAm) with and without PEGDA crosslinker. *AAm/PEGDA* AAm + 10 mass% PEGDA, *PAAm/PEGDA* PAAm + 10 mass% PEGDA, *PAAm* PAAm without PEGDA. Cryogels were formed at a freezing temperature of −20 °C, irradiation time 5 min, and 5 mass% H_2O_2. Reprinted from [16] with permission from Elsevier

irradiated simultaneously. Therefore, the UV irradiation technique is useful for preparation of flat (up to 10 mm), disk-shaped [10–12, 16, 18, 19, 21, 22], and cylindrical cryogels with diameters up to 32 mm [23].

Polymer cryogels obtained by photocrosslinking of frozen systems possess randomly distributed pores [24]. The diffusion of liquids in such cryogels is relatively isotropic (Fig. 13a). In the case of directionally frozen systems, the structure obtained is aligned and the diffusion of liquids through the cryogel follows a one-dimentional pathway, diffusing faster parallel to the direction of freezing (Fig. 13b).

Compression tests performed to determine the aligned cryogel strength revealed that the sample crushed parallel to the freezing direction has a Young's modulus of 10 kPa and the sample crushed in the perpendicular direction has a Young's modulus of 0.9 kPa.

4 Temperature-Responsive Polymer Cryogels

Temperature-responsive polymer cryogels are among the most intriguing representatives of the so-called "inteligent" hydrogels due to their numerous advantages. The hydration/dehydration behavior of cryogels is much more rapid compared to conventional hydrogels obtained from the same polymer [25]. This behavior has

Table 3 Formation of cryogels from monomers via UV irradiation

Monomer precursor	Monomer concentration (mass%)	Irradiation time (min)	Gel fraction yield (%)
NIPAAm	2	5	>99
	5	5	>99
	10	5	97
	15	5	87
AAm	2	5	>99
	5	5	>99
	10	5	96
	15	5	90
ETEGA	2	5	>99
	5	5	>99
	10	5	>99
HEMA	2	5	92
	5	5	92
	10	5	92
	15	5	87
VCL	2	10	–
	5	10	–
	10	10	25
	15	10	15

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Experimental conditions: temperature of freezing −20 °C, 5 mass% H_2O_2, 10 mass% PEGDA (molar mass 575 g/mol)
NIPAAm *N*-isopropylacrylamide, *AAm* acrylamide, *ETEGA* ethoxytriethyleneglycol acrylate, *HEMA* 2-hydroxyethyl methacrylate, *VCL* vinyl caprolactam

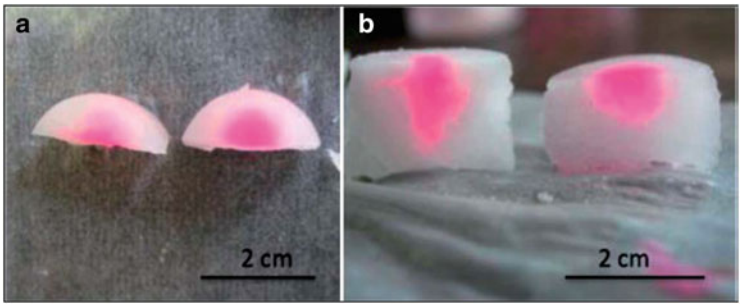

Fig. 13 Diffusion of Rhodamine B-stained water through aligned porous polyOEGMA hydrogel (*left*) and randomly macroporous polyOEGMA hydrogel (*right*) at room temperature: (**a**) birds-eye view, (**b**) cross-sectional view. Reprinted form [24] with permission from the Royal Society of Chemistry

been attributed to the spongy-like structure of the cryogel, which contains a large amount of free water. During hydration/dehydration of the cryogel, the interconnected pores, with their very smooth wall interfaces, dramatically facilitate the diffusion of water and heat exchange.

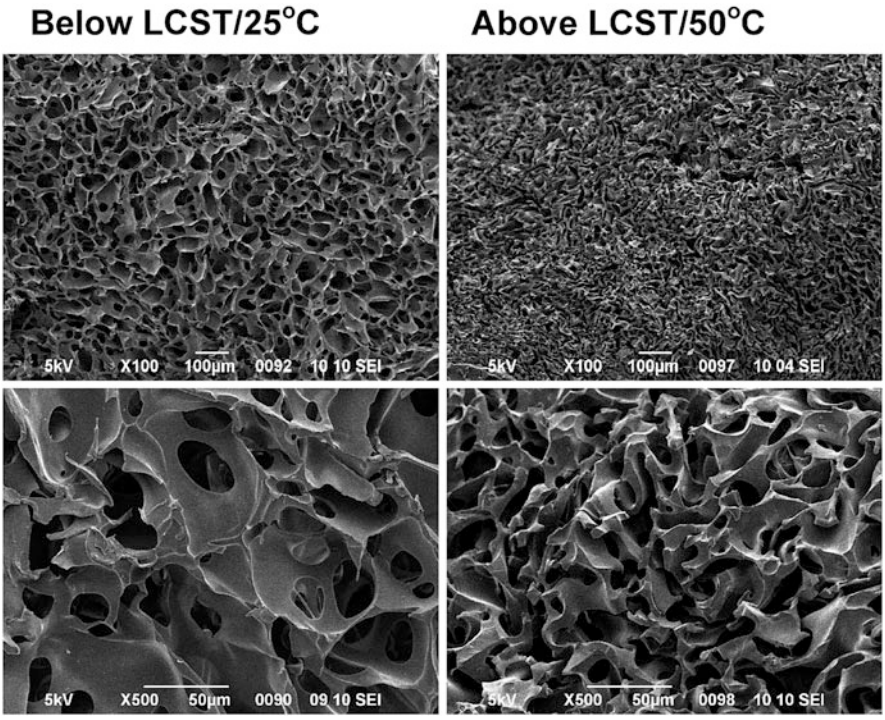

Fig. 14 SEM micrographs of PNIPAAm cryogel prepared from samples immersed in water at 25 °C (*left*) and 50 °C (*right*). Cryogel was synthesized from 5 mass% monomer solution, containing H_2O_2 (5 mass% to monomer) and PEGDA (10 mass%), frozen at −20 °C, and irradiated with UV light for 5 min. Reprinted from [16] with permission from Elsevier

The interior morphologies of PNIPAAm cryogel at temperatures below and above the temperature of volume phase transition (T_{VPT}) are shown in Fig. 14. At 25 °C, the PNIPAAm cryogel is swollen and has a supermacroporous structure with round-shaped interconnected pores (50–100 μm) surrounded by thin walls (ca. 1–2 μm). The phase transition of PNIPAAm caused a drastic decrease in the volume of cryogel, resulting in much smaller pores (Fig. 14, right). Notably, the cryogel does not lose its open porous structure in the deswollen state.

The extremely fast volume phase transition from hydrophilic to hydrophobic state of the polymer network is demonstrated in Fig. 15 for cryogels based on PNIPAAm and hydroxypropylcellulose (HPC). When the temperature changed from 20 to 60 °C, the gel collapsed and reached a near-equilibrium state within 10–12 s. This behavior is attributed to both the existence of a large amount of free water in the pores, which facilitates the heat transfer, and the thin compact cryogel walls, which tend to respond more quickly to temperature changes. The fact that the cryogels preserve their capillary structure above T_{VPT} plays an important role in the rapid transition from hydrophobic to hydrophilic state. As also seen from Fig. 15,

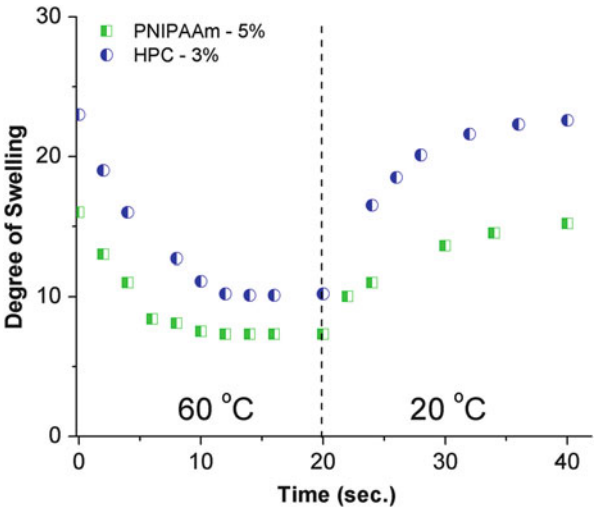

Fig. 15 Deswelling–reswelling kinetics of HPC and PNIPAAm cryogels synthesized via UV irradiation. Reprinted from [16] with permission from Elsevier

the cryogel reached 90 % of the equilibrium degree of swelling at 20 °C within 15–20 s.

The temperature of volume phase transition of each cryogel depends on the nature of the polymer network. The T_{VPT}, estimated as the maximum of the first derivative of the swelling versus temperature curve, of PNIPAAm, HPC, and PETEGA cryogels are 32, 48, and 33 °C, respectively. Most often, T_{VPT} is close to the lower critical solution temperature (LCST) of the linear polymer analog; however, unlike it, the transition is not so sharp.

The swelling versus temperature curves of hydrophobically modified PGL cryogels of different composition are shown in Fig. 16. As a rule, an increase in the degree of modification results in decreased T_{VPT}. Obviously, the gels containing lower amounts of hydrophilic groups need less energy to overcome contributions from the hydrogen bonds formed between the water molecules and polymer network, and the gels collapse at lower temperatures. Thus, one may design hydrophobically modified PGL cryogels with the desired T_{VPT} just by tuning the degree of modification of the precursor. It should be noted that changing the concentration of the copolymer solutions studied (1–5 mass%) did not cause any shift of T_{VPT}.

The ability of stimuli-responsive hydrogels to undergo a reversible volume phase transition in a defined and reproducible manner under given environmental parameters is a crucial precondition for their practical application. Multiple swelling–deswelling experiments conducted with PNIPAAm, PETEGA, and HPC cryogels at different temperatures revealed nearly equal degrees of swelling for each material at a defined temperature. This indicates that the cryogel is able to reproduce its former interior structure and volume after many cycles of volume phase transition without any mechanical destruction. An exception was observed only for hydrophobically modified PGL cryogels. A decrease in the degree of

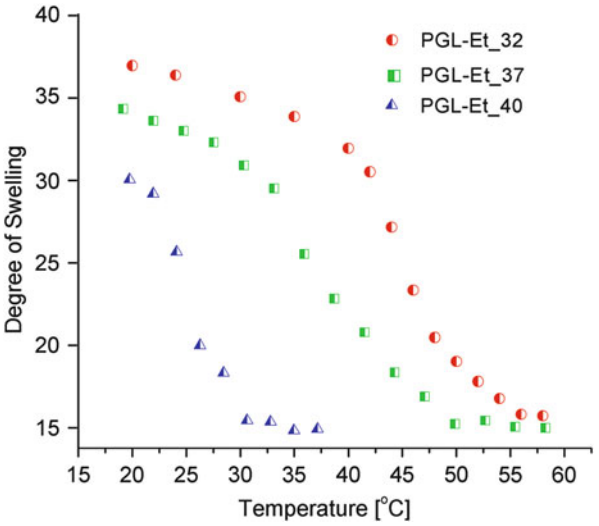

Fig. 16 Temperature dependence of the swelling degree of different hydrophobically modified PGL cryogels (degree of modification 32, 37, and 40 mol%) obtained at a freezing temperature of −20 °C, initial polymer concentration 3 mass%, 5 mass% BBTMAC, irradiation time 4 min. Reprinted from [19] with permission from Elsevier

swelling was found after the fifth cycle of temperature changes and was caused by separation of small pieces from the edge of the cryogel. This destruction is attributed to the lower strength of ether bonds in the PGL network upon mechanical deformation accompanied by the temperature changes.

5 Nanocomposites Based on Polymer Cryogels

Fabrication of nanocomposite cryogels is a versatile platform for imparting specific properties to the material. Basically, the specific heterogeneous structure of the cryogel makes possible the incorporation of nanosized fillers via two different routes: into the cryogel walls and into the cryogel channels. This, however, may lead to nanocomposite cryogels with different behaviors and properties, even though they were prepared from the same initial components. For instance, two types of HPC cryogels containing silver nanoparticles (AgNPs), either entrapped into the gel walls (polymer matrix) or included into the pores, were synthesized via UV-induced crosslinking [16]. AgNPs were immobilized in the channels of the gel by immersing a pre-made freeze-dried HPC cryogel in an aqueous dispersion of AgNPs. The freeze-drying process preserves the spongy-like macroporous structure of materials and, thus, the dispersion can easily fill the channels (interconnected pores) of the dry cryogel in a few seconds. The second approach is based on mixing of AgNPs and polymer prior to freezing and crosslinking. Cryogenic treatment led to phase-separated ice crystals and nonfrozen liquid microphase containing AgNPs, reagents, and physically bound water. Because the reaction of crosslinking occurs only in the liquid microphase forming the gel walls, AgNPs were embedded into the crosslinked polymer matrix. It is noteworthy that both types of materials exhibited

completely different properties. The cryogels containing AgNPs only in the channels released them immediately when compressed (Fig. 17a) or, in the case of temperature-responsive polymers, by switching to temperatures above the corresponding T_{VPT} [16]. In addition, placed in a large excess of water, the cryogels released the particles within several hours without external stimuli. In contrast, the cryogels with AgNPs embedded in the walls did not release any nanoparticles (Fig. 17b) due to the dense polymer network. In the latter case, only a slow release of Ag^+ was registered.

Supermacroporous carbon nanotube (CNT)–polymer nanocomposites based on various polymer cryogels have been prepared via photocrosslinking of either polymer or monomer precursors [26, 27]. The two different strategies described above were exploited to fabricate foam-like materials (aerogels) with CNTs located either in the cryogel walls or on the cryogel inner surface (Fig. 18).

Intererestingly, the inclusion of a CNT dispersion into the pores of a pre-made cryogel and the subsequent freezing resulted in deposition of both single- and multiwalled CNTs onto the inner surface of the polymer matrix. It is assumed that during cryogenic treatment most of the water in the system forms ice crystals, whereas CNTs are accumulated on the surface of crystals and are gradually pushed to the cryogel walls. Based on this original technique, supermacroporous aerogels of high electrical conductivity at relatively low CNT content (0.12 mass%) were obtained. Specifically for the UV irradiation technique, when the nanotubes were added into the system before crosslinking, a decrease in the gel fraction yield was found. Such a result is attributed to the ability of CNTs to absorb UV light, which interferes with the regular crosslinking of polymer matrix, thus yielding cryogels with a lower mechanical strength than the pure cryogels from the same precursor.

6 Applications

One of the main applications of polymer cryogels is their use in biotechnology as carriers of cells, bacteria, and enzymes [1, 2]. The advantages of immobilized microorganisms over cultures in suspension include the easier collection and purification of bioproducts, better stability and performance under storage and operational conditions, tolerance against toxic compounds, etc.

Photocrosslinked HEC cryogels have been studied as matrices for immobilization of *Saccharomyces cerevisiae* cells [28, 29]. The systems obtained can be re-used many times for production of ethanol in a batch reactor. Even after 6 months of storage, *S. cerevisiae* cells were able to produce over 40 g ethanol in 1 L reactor volume. However, the larger pore size of cryogels as compared to the cell size allows unhindered difusion of the cells located at the periphery of the polymer matrix into the medium. Therefore, a mixed system is formed consisting of immobilized and free cells. The leakage of cells was significantly reduced by covering the HEC cryogel, containing *S. cerevisiae* cells, with an outer layer based on photocrosslinked poly(ethylene oxide) (PEO) hydrogel [30]. The PEO

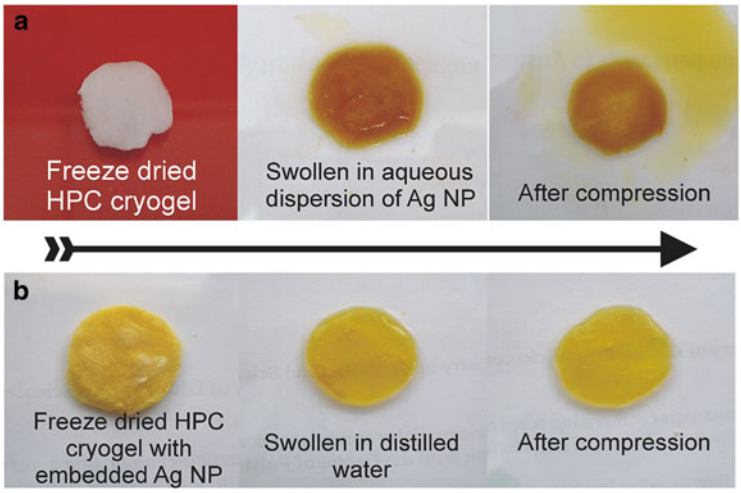

Fig. 17 Nanocomposite polymer cryogels based on HPC and silver nanoparticles immobilized into (**a**) the pores and (**b**) the walls of the cryogel. Reprinted from [16] with permission from Elsevier

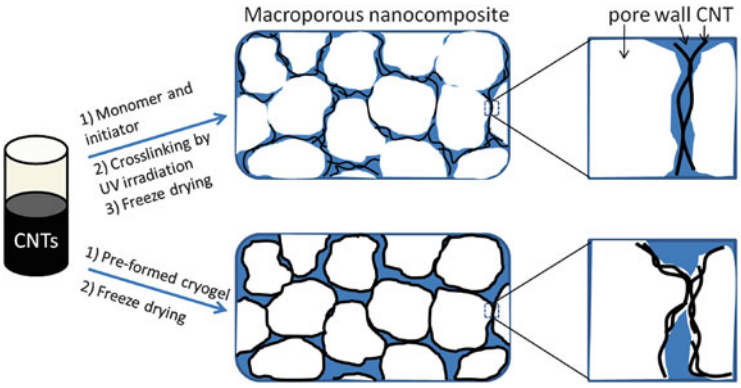

Fig. 18 Fabrication of CNT–cryogel composites with nanotubes embedded into the cryogel walls (*top*) and nanotubes deposited onto the inner surface of the cryogel (*bottom*). Reprinted from [27] with permission from Elsevier

layer did not hinder the diffusion of nutrient and fermentation products. In this case, ethanol production was up to 77 % of the theoretical yield.

Photocrosslinked PEO cryogel was investigated as carrier for different xenobiotic-degrading bacteria [31, 32]. The cryogel exhibited good mechanical strength, nontoxicity, and high biocompatibility with bacteria. It was found that the enzyme activity of immobilized bacteria is many times higher than that of the free culture.

Production of a rhamnolipid biosurfactant by cells of *Pseudomonas aeruginosa* strain BN10 immobilized into PEO and PAAm cryogels was investigated under semicontinuous shake flask conditions and compared to biosurfactant secretion by free cells [33]. The yield of rhamnolipids in the immobilized system exceeded that of the free bacterial cells, distinguishing an effective bioprocess. The polymer matrices possessed chemical and biological stability and very good physico-mechanical characteristics, which are prerequisites for a high life span of these materials for the production of rhamnolipids.

The temperature-responsive properties of poly(glycidol-*co*-ethyl glycidyl carbamate) cryogels were exploited for the growth of fibroblast cells on their surface [19]. It was shown that the nonionic hydrophilic surface hinders interactions with cells. When the environmental temperature is above T_{VPT} of the cryogel, the support became hydrophobic, allowing cell attachment and proliferation.

Supermacroporous PNIPAAm cryogels containing urease were prepared via the UV irradiation technique, with hydrogen peroxide as initiator [34]. Taking the advantage of cryostructuration phenomenon during the cryogenic treatment, most enzyme molecules were embedded into the cryogel walls. Although the enzyme was physically entrapped, the system exhibited remarkable resistance against leakage due to the dense polymer network formed in the cryogel walls. The immobilized urease can catalyze the hydrolysis of urea over a broad temperature range in both batch and flow regimes. The interconnected macropores assist unhindered diffusion of the substrate and reaction products through the gel, thus paving the way for consecutive re-use at a constant activity, in contrast to the conventional PNIPAAm hydrogel (Fig. 19). The relatively high flow rate through the cryogel matrix and the good activity of urease make it possible to directly remove urea from the feed solution in a continuous flow regime. Hence, this material might be attractive for treatment of contaminated water, blood detoxication, the dialysate regeneration system of artificial kidneys, removal of urea from beverages, etc.

Encapsulation of a high amount of enzyme, comparable to the weight of cryogel matrix, is only possible when the enzyme solution fills the large volume pores of a pre-made cryogel. In this case, the performance of the system depends strongly on the enzyme retention. It was demonstrated that the fabrication of an outer PEO layer onto a PHEMA cryogel containing urease in the pores (Fig. 20) is an effective strategy for preventing leakage of enzyme into the medium [35]. Moreover, one could expect that the urease molecules, simply located in the confined space of the macropores, are not restricted and behave like the free enzyme molecules. Due to the high sensitivity of urease, such a system is able to detect very low contamination of metal ions in water and, therefore, can find application as a biosensor.

One important issue in modern medicine is linked to the controlled release of active substances. It is assumed that a drug delivery system can supply a constant dose of the active substance over a period of several hours or days, depending on the particular disease. Thus, it has an advantage over the conventional system, characterized by a spontaneous release of the active substance immediately after administration (known as the “burst” effect) [36]. The potential of polymer cryogels for

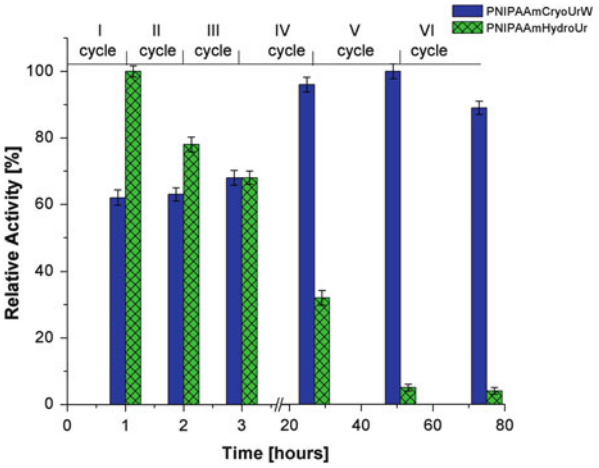

Fig. 19 Effect of reaction time and number of cycles on the enzymatic activity of urease immobilized in PNIPAAm cryogel and PNIPAAm hydrogel, incubated in 10 mL urea solution (3.2 g/L) at 50 °C. Reprinted from [34] with permission from John Wiley & Sons

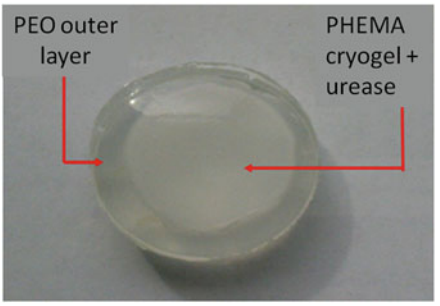

Fig. 20 Digital image of double-layered gel comprising a PHEMA core containing urease and a PEO outer layer. Reprinted from [35] with permission from John Wiley & Sons

encapsulation and sustained release of hydrophilic drugs has been investigated [21, 22]. By design, the water-soluble drug verapamil hydrochloride was entrapped in the walls of different cryogels. In vitro experiments showed that the swelling behavior of polymer matrices at physiological temperature plays a key role in the drug release profile. For instance, the temperature-responsive PETEGA (Fig. 21) and PNIPAAm cryogels, which are in a hydrophobic state at 37 °C, released the drug over a period of more than 8 h. Following a slight initial burst effect, from the second hour post-incubation the drug release process is characterized by time-independent kinetics (zero-order kinetics with correlation coefficient $R = 0.998$). This sustained release is attributed to the hindered diffusion of drug molecules across the deswollen polymer network.

On the other hand, drug delivery systems based on hydrophilic PHEMA or HEC–chitosan cryogels exhibited prolonged drug release at 37 °C after incorporation of a high amount of crosslinking agent (30 mass%) into the polymer network [19, 22]. It is suggested that the high crosslink density of the polymer network is responsible for the reduced rate of diffusion of drug molecules.

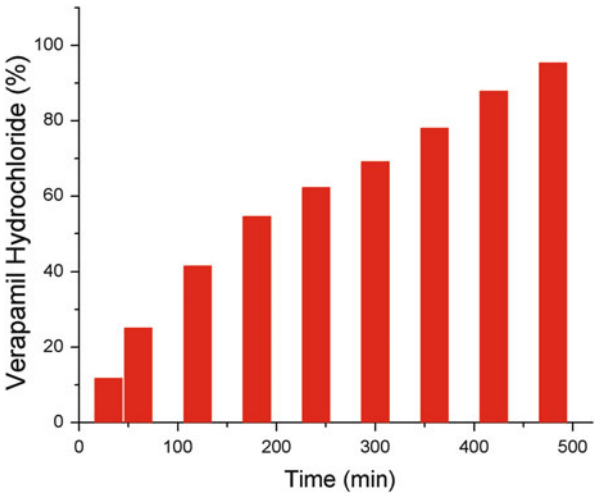

Fig. 21 Release kinetics of verapamil hydrochloride from PETEGA cryogel at 37 °C. Reproduced from [22] with permission from Elsevier

7 Conclusions

The combination of cryogenic treatment and UV irradiation is a facile method for the synthesis of chemically crosslinked supermacroporous polymer cryogels from both polymer and monomer precursors. Effective crosslinking and a high gel fraction yield can be achieved by irradiation of partly frozen systems with a UV curing equipment comprising a 400 W metal halide flood lamp emitting full-spectrum UV–visible light at an irradiation dose rate of 5.7 J/cm^2 min (input power 93 mW/cm^2) for 2–5 min. The cryogels obtained possess an open porous structure, which imparts a very rapid water uptake and, in the case of stimuli-responsive polymers, reversible ultrarapid volume phase transition.

Cryogels of cellulose derivatives were successfully synthesized by UV irradiation of their partly frozen solutions. The use of H_2O_2 initiator for photocrosslinking of biocompatible and biodegradable polymers allows preparation of "green" gel materials that can be further exploited without any additional purification.

In general, different species such as water-soluble drugs, enzymes, nanoparticles, and nanotubes can be immobilized either within the cryogel walls or into the cryogel pores, depending on the experimental protocol. Hence, one can fabricate materials with specific properties, for instance, drug carriers exhibiting either fast or prolonged release profiles.

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