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Versatility of the Preparation Method for Macroporous Cryogel Particles Utilizing the Inverse Leidenfrost Effect

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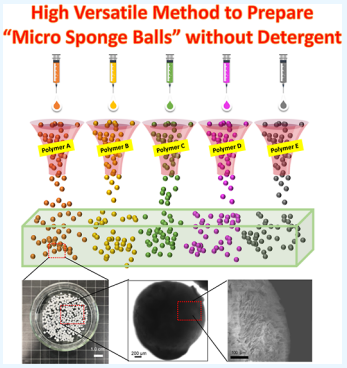
ABSTRACT: We have investigated the versatility of a two-step preparation method, without a detergent, that combines both the inverse Leidenfrost effect and the cryogelation technique by using the macroporous particles of different kinds of monomers (four vinyl monomers) or a natural polymer (agarose). First, the precursor of polymers was dropped into liquid nitrogen to prepare the spherical frozen droplet by the inverse Leidenfrost effect. Second, the frozen droplets were cryo-polymerized at the frozen temperature; then, cryogel particles were prepared after thawing. Subsequently, the basic characteristics of the macroporous polymer particles obtained above were compared, focusing on the appearances, porous morphologies, and mechanical properties. It was found that the similar polymer particles could be obtained by the two-step preparation method, while there was a slight difference in their characteristics, depending on the type of monomers. Especially for the mechanical properties, the cryogel particles of the hydrophilic polymer exhibited a shape memory function with sponge-like elasticity, whereas the hydrophobic polymer particles were observed to be cracked after compression (i.e., no shape memory function). This work provides a versatile method of adopting various kinds of monomers and natural polymers for the preparation of macroporous particles. Hence, the method possibly has a potential to prepare and design "tailor-made" macroporous polymer particles for the application purpose.



INTRODUCTION

Porous polymer particles are useful materials to apply for various fields, such as sensor materials, basal particular materials for separation and purification, culture matrixes for tissue engineering, and so on.[1−5] The porous polymer particles have a large specific surface area due to porous morphologies (i.e., macro-porous structures: >50 nm, meso-porous structures: 2−50 nm, and micro-porous structures: <2 nm), which could contribute to the efficient diffusion of the target molecules in the case of separation and purification processes. Furthermore, it is known that the porous polymer particles possess some superior physical properties, for instance, durability, stretchability, and elasticity.[6−8] In addition, the porous polymer particles could also be utilized for the immobilization of self-assemblies, which usually exist as a "dispersed" state in water, such as liposomes/vesicles,[9,10] colloids,[11] and supermolecules,[12] resulting in the utilization of the specific functions of their self-assemblies in a separation process design. From another point of view, the macro-/micro-porous polymer particles are expected to be applied as functional supports for various kinds of separation by modifying their surface by active and functional molecules.[13] In general, an emulsion polymerization method is commonly adopted for the preparation strategy of porous polymer particles.[14] In general, during the polymerization process, detergents were required for the stabilization of emulsion in order to use them as a mold of the particle shape; the detergent molecules were adsorbed at the interface between the continuous phase and dispersed phase, and the emulsion therefore exists stably in the bulk phase during the polymerization process.[15] However, there is a concern that detergents themselves may affect the performance of porous polymer particles. The remaining detergents retained on the particle may negatively interfere with the characteristics of the polymer surface modified with the foreign molecules.

In order to overcome the above-mentioned problem, it is important to establish a "detergent-free" method for the preparation of porous polymer particles, which enables us to prepare a variety of polymer particles regardless of the polymer characters. Cryogels are a unique porous materials that are known to have monolithic supermacropores (10−100 μm).[16] They are generally produced by polymerization of the polymer precursor solution at the frozen temperature point of the solvent (i.e., water).[17] During the cryo-polymerization step, a phase separation occurs between solvent crystals and the

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concentrated polymer precursor (i.e., frozen phase and unfrozen liquid micro phase).[18] As a result, the crystals are deposited as the porogen, and the cryogel forms large porous structures after polymerization and thawing.[19] Here, the properties of the cryogel can be easily controlled by the polymerization condition (e.g., monomer/polymer component, polymerization temperature, and amount ratio of the monomer and solvent). In addition to the beneficial aspect of various properties of the cryogels being flexibly designed, the shape of cryogel particles is difficult to be controlled without the use of a detergent, where the emulsion without any detergent for the cryo-polymerization at the frozen temperature becomes unstable, and therefore, the colloidal coalescence occurs.[20] We have recently reported the novel preparation method via a two-step process for cryogel particles without any detergent, where (1) the inverse-Leidenfrost effect and (2) the cryo-polymerization method were adopted stepwisely.[21] The polymer particles with spherical shapes can be easily formed on the liquid nitrogen because the boiling film coats the surface of the water droplet including polymer precursors and the included water droplets contribute to the formation of the macroporous structure in the polymer particle via cryo-polymerization.

In this study, we investigated the versatility of the two-step preparation method, without a detergent, that combines both the inverse Leidenfrost effect and the cryo-gelation technique by preparing the macroporous particles of different kinds of monomers (four vinyl monomers that differ in the structure and physicochemical character) or a natural polymer (agarose). Subsequently, the prepared cryogel particles were systematically characterized, focusing on the (i) macroscopic structures [particle shape and size by using a charge coupled device (CCD) camera optical microscope], (ii) microscopic structures (porous and monolithic structures by using a scanning electron microscope), and (iii) mechanical properties (elasticity and shape memory by using the CCD and a piston device). The results obtained were finally compared to discuss the versatility of the proposed methodology.

MATERIALS

Acrylamide (AAm), *N*,*N*′-methylenebisacrylamide (MBA), *N*-isopropylacrylamide (NIPA), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), glutaraldehyde solution (25%), gelatin, agarose XP (a low melting point of ≤ 65 °C and a gelling temperature of ≤ 30 °C), liquid paraffine, acetic acid, and ethanol were purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan). Ultra-pure water (conductivity 18.2 MΩ cm) was prepared using Direct-Q UV 3 (Merch, Osaka, Japan). Trimethylolpropane trimethacrylate (Trim), benzoyl peroxide (BPO), and ethyl-4-dimethylaminobenzoate (EDMAB) were purchased from Tokyo Chemical Industry Ltd. All materials were used as received without further treatment.

METHOD

Preparation of Cryogel Particles. Briefly, cryogel particles were prepared harnessing the technique reported in our previous research. Cryogel particles were synthesized via a two-step process: (1) preparation of frozen droplets by the inverse Leidenfrost effect and (2) cryo-gelation by frozen polymerization. The spherical shape of the cryogel particles was formed by the inverse Leidenfrost effect. When droplets approached an extremely cold substrate such as liquid nitrogen (−196 °C), they were coated by boiling films and formed into the spherical shape frozen droplets.

Cryogel Particles. Our method for the preparation of cryogel particles enables easy design/control of their properties. By combining different types of monomers and cross-linkers, cryogel particles based on different kinds of monomers or a natural polymer (agarose) were prepared: (a) AAm, (b) NIPA, (c) 2-hydroxyethylmethacrylate (HEMA), (d) Trim, and (e) agarose. The AAm-based cryogel particles were prepared via the process: 20 mmol AAm and 3.2 mmol MBA were dissolved in 40 mL of ultra-pure water and degassed with N_2 gas for 15 min. After that, 1 mM APS and 60 μL of TEMED were added into the solution. Then, the obtained solution was dropped into liquid nitrogen using a syringe, and the prepared frozen droplets contained the polymer precursor. With the same operations, the NIPAm-based cryogel particles were prepared. 20 mmol NIPAm, 3.2 mmol MBA, 1 mM APS, and 60 μL of TEMED were used. Next, the Trim-based cryogel particles were prepared. 29 mmol Trim and 2.4 mmol MBA were dissolved in 25 mL of acetic acid. After that, 3 mmol BPO and 2.9 mmol EDMAB were added into the solution. Then, the obtained solution was dropped into liquid nitrogen using a syringe. All different types of frozen droplets were transferred to liquid paraffine (−15 °C), and cryo-polymerization was started overnight.

Shape of Cryogel Particles. All the different based cryogel particles were observed using an optical microscope (BX53, Olympus, Tokyo, Japan), with a ×4 objective lens and a CCD camera. The particles sizes and sphericities were measured from optical microscopy images. The sphericity of cryogel particles (S) is defined in the following eq 1

$$S[\%] = r/R \times 100 \quad (1)$$

where R and r represent the maximum and minimum diameters of cryogel particles, respectively. The particles sizes are measured from r.

Morphology of Cryogel Particles. The porous structure of cryogel particles was observed using a scanning electron microscope, operated at 20 keV. As a pretreatment, all cryogel particles were dried by lyophilization and coated with gold for 30 s by spattering. The surface section and cross-section of cryogel particles were observed as shown in Figure S1. The porosity of cryogel particles were calculated from scanning electron microscopy (SEM) images by using Image J software.

Mechanical Compression Test. Mechanical properties of cryogel particles were confirmed by a mechanical compression−release cycle. The experimental setup shown in Figure S2 was prepared. The obtained cryogel particle was inserted into a microsyringe (Hamilton, 500 μL). Subsequently, the shape of the particle in the microsyringe was observed using an optical microscope with a ×4 objective lens. While the particle in Figure 2 shows an elliptical shape due to the refraction on the surface of a cylindrical shape of the microsyringe, we confirmed that the shape of the particle is spherical before and after the particles were placed in the syringe without compression.

RESULTS AND DISCUSSION

The properties of polymer materials are known to be dependent on the type of physicochemical natures of their building block. Hence, the choice of the monomers for the synthetic polymer and cross-linker and the natural polymer itself is a key factor in obtaining the appropriate materials for

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Table 1. Summary of Experimental Conditions of the Preparation of Different Polymer Particles and Their Physical Characteristics

monomer (cross-linker)	(a) AAm (MBA)	(b) NIPA (MBA)	(c) HEMA (MBA)	(d) Trim (MBA)	(e) agarose (gelatin)
1. name	acrylamide	NIPA	2-hydroxyethyl-methacrylate	Trim	agarose
2. cross-linking	chemical cross-linking	chemical cross-linking	chemical cross-linking	chemical cross-linking	physical cross-linking
3. hydrophilicity	hydrophilic	hydrophilic	hydrophilic	hydrophobic	hydrophilic
4. porogen	ice crystals	ice crystals	ice crystals	acetic acid crystals	ice crystals/acetic acid
5. particle size [μm]	1807.3	1682.8	1490.4	1599.5	2721.5
6. sphericity [%]	95.2	96.0	92.9	93.4	96.5
7. porosity [%]	88.4	79.2	82.3	74.3	81.0
8. elasticity	positive	positive	positive	negative	positive

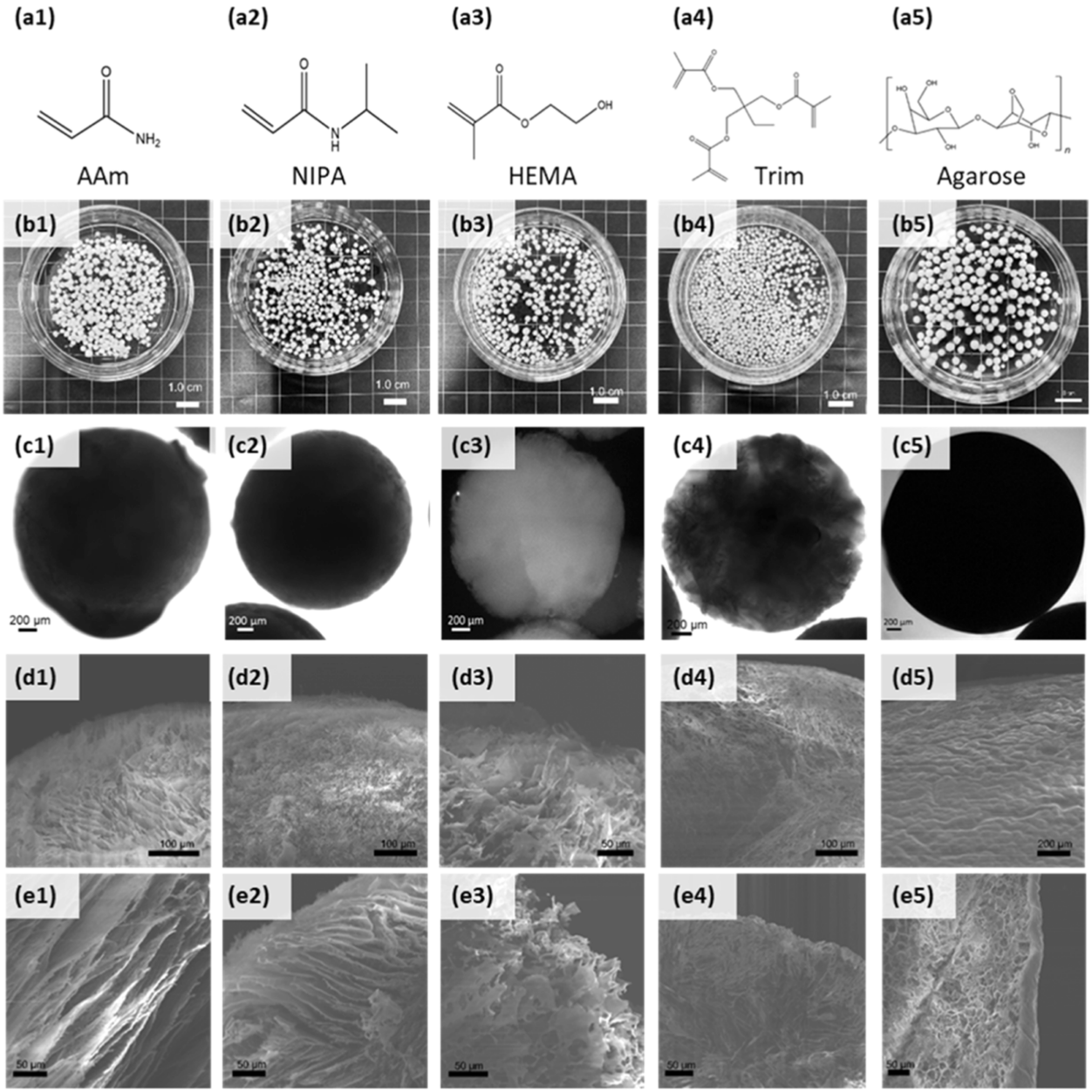

Figure 1. Structures of polymer particles obtained by cryo-polymerization technique combined with the inverse Leidenfrost effect. (a1−a4) Chemical structure of different monomers and (a5) basal unit structure of agarose. (b1−b5) CCD images of obtained polymer particles. (c1−c5) Microscopic images of obtained polymers particles. (d1−d5) SEM images of the surface section of the polymer particles. (e1−e5) SEM images of the cross-section of the polymer particles.

their specific application. We prepared five kinds of cryogel particles by selecting four synthetic polymers with different monomers and a natural polymer as case studies in order to prove the versatility of our previous method including the two-

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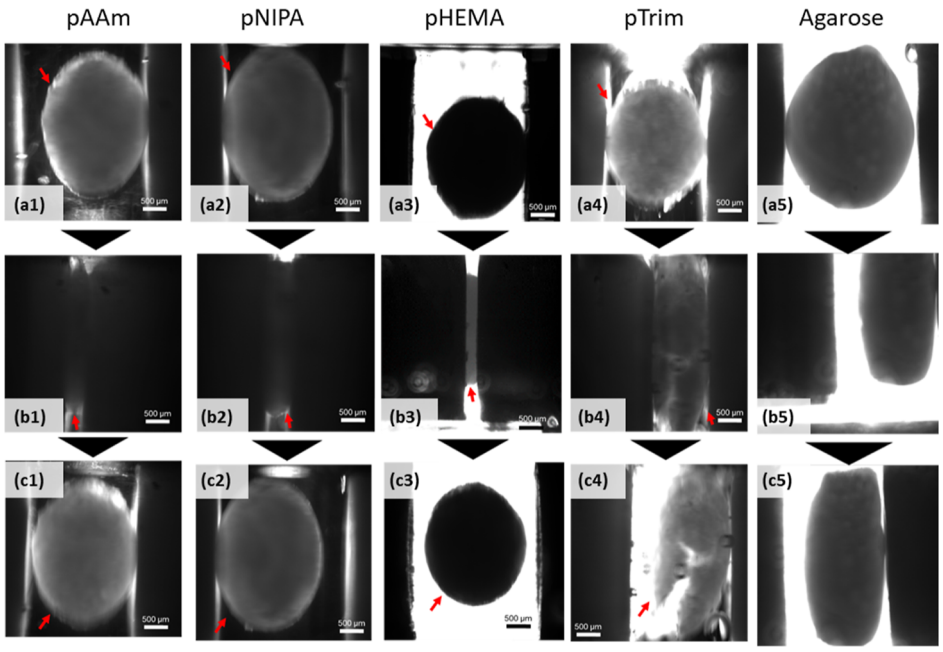

Figure 2. Mechanical properties of cryogel particles under compression and release of the polymer particles in a microsyringe. Optical microscopy images of cryogel particles: before compression (a1−a5), during compression (b1−b5), and after compression(c1−c5).

step preparation process.[21] All the experimental conditions and results for the polymer particles are summarized in Table 1 and Figures 1 and 2, together with the Supporting Information.

The chemical structures and natures of the monomers and polymers used here are summarized in the top lines (first to third lines) of Table 1 and Figure 1(a1−a5). In relation to the synthetic polymers, hydrophilic monomers, such as (a) AAm and (b) NIPA, are used for the polymer particle preparation, where AAm has a primary amide group (−$COONH_2$) and NIPA has an amide group (−CO−NH−) as the branched group. NIPA also has an isopropyl group [−$CH(CH_3)_2$]. (c) HEMA monomer is also selected as a "hydrophilic" monomer that possesses a hydroxyethyl functional group (−CH_2−CH_2−OH), an ester group (−COO−), and an acrylic functional group. (d) Trim is selected because of its "hydrophobic" nature. There are only a few reports on the preparation of hydrophobic cryogels.[22] Trim has three hydrophobic methacrylic groups that are conjugated with the "hydrophobic" and "branched" trimethyl propanyl group in its monomer structure, and mechanically tough polymer materials are known to be obtained after its polymerization. (e) Agarose is known as a natural polymer. The chemical structure of a minimal unit of agarose is a disaccharide, and the unit of alternating 1−4 linked 3,6-anhydro-α-L-galactopyranose and 1,3-linked β-D-galactopyranose is repeated in a polymer.[23] The properties of polymer materials are also varied depending on the type of cross-linking (i.e., chemical and physical cross-linking). The synthesized polymer of cryogel particles based on AAm, NIPA, HEMA, and Trim were cross-linked with MBA via chemical cross-linking, whereas the cryogel particles of a natural polymer, agarose, were cross-linked with gelatin via physical cross-linking. The role of the porogen is important in preparing porous materials. During the cryo-polymerization, the frozen region (solvent crystals) and the non-frozen region (concentrated precursor) are formed as a result of the phase separation. The type of the porogen is shown in the fourth line of Table 1. The solvents such as water and acetic acid used for resolving monomers/natural polymers in an aqueous solution become solvent crystals under the frozen condition and act as the porogen. Hence, the ice crystals were the porogen of hydrophilic cryogel particles, whereby the acetic acid crystals were the ones for the preparation of "hydrophobic" cryogel particles.

Physical characters of the polymer particles obtained above are summarized in Figure 1. The appearance of cryogel particles prepared via our preparation method were observed by using a CCD camera, as shown in the images [Figure 1(c1−c5)]. All the particles based on different monomers were opaque, implying that cryogel particles have a porous structure and the light was scattered.[24] The pAAm, pNIPA, pHEMA, and pTrim cryogel particles were white in color, whereas agarose cryogel particles were yellow. Subsequently, the shapes and sizes of the cryogel particle were observed by using an optical microscope, as shown in the images (the sixth line, Table 1). Average diameters of the particle were around 1600−1800 μm with high sphericities (93.4−96.0%) (the seventh line, Table 1). Herewith, only agarose cryogel particles have a relatively large diameter. In this preparation process, the diameter of the particles was dependent on droplet volume (V), and it was calculated from eq 2.[25]

$$V = d_0\pi\sigma/\rho g \tag{2}$$

where d_0 is the needle's interior diameter, σ and ρ represent the surface tension and the density of the solution, respectively, and g is the gravitational force. Herein, the agarose solution during the preparation process has high viscosity as compared to the other type of monomer solution. As a result, the σ value increases and the V becomes large, resulting in the diameter of the agarose cryogel becoming larger. Moreover, according to eq 2, the diameter of particles is possibly controlled by the needle's interior diameter (data not shown).

The cryogel is known to have a unique porous structure called as the "supermacroporous structure" in the range of 10−200 μm pore size. The morphologies were observed by using

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SEM on surfaces and cross-sections of cryogel particles. The obtained images of the surface of the polymer particles are summarized in Figure 1(d1−d5). It was found that the large pores were distributed on the surface section of almost all cryogel particles, except for agarose cryogel particles [Figure 1(d5)]. Subsequently, the cross-section of cryogel particles was also observed by SEM [Figure 1 (e1−e5)], indicating that all the cryogel particles have a supermacroporous structure with high porosity. Interestingly, the porous structure of the agarose cryogel particle was found to be different between the surface and cross-section; the surface is smooth with less pores, and on the other hand, the interior still maintains its porous structure. The formation of this difference in the porous morphology between the surface and internal region was due to the high viscosity of the precursor.[23] The shear stress works at the fluid interface.[26] During the preparation process, when the polymer precursor droplet was coated with a boiling film of liquid nitrogen by the inverse Leidenfrost effect, the shear stress would work at the interface between the droplet and nitrogen gas. As a result, agarose cryogel particles have a specific surface texture with a smooth appearance. However, there is a limitation for shear stress to reach deep inside the particles due to the high viscosity of the precursor, and the macroporous structure could be maintained at the interior. In contrast, the solutions of other polymer precursors (i.e., AAm, NIPA, HEMA, and Trim) have low viscosity as compared with the agarose precursor solution; therefore, these systems would not experience shear stress on the surface of the droplet, which results in the porous structure being uniform throughout the particle.

The mechanical properties of materials are an important factor for application such as separation and purification technologies. Herein, the mechanical property of cryogel particles was investigated with the compression and release operation in a microsyringe. The images of "before compression" "during compression", and "after compression" could be used to discuss the shape memory properties. The obtained results are summarized in Figure 2. The cryogel particles of pAAm, pNIPA, and pHEMA were confirmed to show sponge-like elasticity (eighth line in Table 1). After the shape of these particles was deformed during compression, it was restored rapidly after the release of the mechanical force [from (a) to (c) for each polymer particle in Figure 1]. On the contrary, the hydrophobic cryogel particle of pTrim indicated a different mechanical behavior: mechanical compression was not reversible (13th line). The pTrim cryogel particle was cracked, and after the release of the mechanical force, it was not restored to the original shape. This is possibly because part of the three acrylic groups of Trim could work as a cross-linker and, then, a high-density polymer wall could be formed, resulting in the mechanical property of the polymer being kept tough. Hence, the decrease of entropic elasticity was probably caused by the side groups of the polymer backbone, showing that the particle is revealed to be tough and does not have sponge-like elasticity. This is the first report on the preparation of hydrophobic cryogel particles of pTrim. As shown in (a5), (b5), and (c5) in Figure 2, the agarose cryogel particle was found to show an interesting mechanical behavior. Although agarose cryogel particles exhibited an elastic property, their mechanical property was different from that of other hydrophilic cryogel particles. The particle was slightly hard as compared with synthetic polymers, and after the release of mechanical compression, it took several minutes to restore the shape (data not shown). Furthermore, the particle was cracked upon a strong mechanical impact. This property is considered to be caused by the gradient porosity of agarose cryogel particles. Since the surface of agarose cryogel particles does not have well-distributed pores, water diffusion into the interior of the particles is low. Hence, it takes a relatively longer time for the particles to restore their shape after compression. The structure of agarose cryogel particles can be expected to be applicable as functional particles that enable us to control sustain-released materials.

CONCLUSIONS

Five types of cryogel particles, which differ in physicochemical properties of polymers, were successfully prepared by using our previous method, without the use of a detergent, which combines the (i) inverse Leidenfrost effect method for the preparation of frozen droplets containing the precursor and (ii) cryo-polymerization method for polymerization at the frozen temperature. In all cases of hydrophilic and hydrophobic polymers, spherical polymer particles with macroporous structures were found to be successfully prepared, while there are some slight differences in the detailed characters. The hydrophilic polymer particles prepared, such as pAAm, pNIPA, pHEMA, and agarose, show shape memory properties, while hydrophobic polymer particles of pTrim do not. As a whole, this method was found to be versatile and could possibly be beneficial for the "*tailor-made*" preparation of porous polymer particles because there are no limitation caused by detergents for emulsion formation and could also be applied in chemical engineering (i.e., separation materials) and biomedical engineering (i.e., tissue engineering matrix).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06197.

Schematic illustration of the SEM observation position for the surface and cross-section of the cryogel particle and schematic illustration of the mechanical compression test for cryogel particles in a microsyringe, including the state before the compression, state during the compression, and state after the compression (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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