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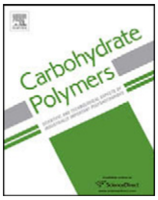

Synthesis of a potato starch-based concentrating polymer and its application in the concentration of extraction solutions

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ABSTRACT

Superabsorbent polymers were prepared with different combinations of reagents, and a potato starch-based polymer was synthesized for concentrating extraction solutions. This polymer was composed of potato starch:water:NaOH:acrylic acid (70% neutralized):$K_2S_2O_8$:glycerol at a ratio of 4:40:1.5:28:0.05:0.45, and absorbed water at up to 767.3, 211.7 and 47.0–59.0 times its own weight in distilled water, bovine serum albumin solution and 0.9% NaCl or extraction solutions, respectively. The polymer can be used for concentrating both macromolecular and small-molecule solutions, with recovery rates of 90% or higher after concentration to 1/3 of the original volume. The results demonstrate that the described polymer can be used for the simple and efficient concentration of extract solutions at room or low temperatures with low energy cost, particularly useful for temperature-sensitive substances.

1. Introduction

The concentration of solutions is a difficult step in the process of extracting and separating bioactive substances from biological materials. Several methods, including precipitation, evaporation, freezing concentration, membrane concentration and chromatographic absorption, have been used in industries and laboratories. These methods require high temperatures, large amounts of energy, considerably long timeframes, membrane blocking and cleaning or high equipment costs. Such methods may also cause the deactivation or degradation of the target substance, particularly when in water-based solutions (Cassano et al., 2003).

Superabsorbent polymers are crosslinked, polymerized resins capable of absorbing and retaining extensive volumes of solvents. They are biodegradable and can be produced with natural macromolecular materials, such as starch, cellulose or plant residues, at low cost (Fernández-Barbero et al., 2009; Liu, Miao, Wang, & Yin, 2009; Teli & Waghmare, 2009). Since the first superabsorbent polymer was reported by Weaver, Bagley, Fanta, and Doane (1976), similar polymers have received significant attention and have been widely used in many areas, such as sanitary goods (Kosemund et al., 2009), agriculture and horticulture (Ni, Liu, & Lu, 2009; Puoci et al., 2008), waste-water treatment (Davies, Novais, & Martins-Dias, 2004; Kaşgöz & Durmus, 2008) and medical drug delivery systems (Cutting, 2009; Fernández-Barbero et al., 2009). In addition to the use of hydrophilic polymers for water swelling, Ono, Sugimoto, Shinkai, and Sada (2007) reported the synthesis of lipophilic superabsorbent polymers bearing tetra-alkylammonium tetraphenylborate as a lipophilic, bulky ionic group. These polymers can absorb up to 500 times their own weight in nonpolar organic solvents, which expands the potential application of these polymers in lipophilic solutions.

Similar to Sephadex G-25 gel, but with a much higher absorption capacity and a much lower cost, the crosslinked structure of superabsorbent polymers makes them capable of absorbing large quantities of water and small solutes, while excluding larger molecules such as proteins. They have been tested for many protein concentration applications (Badiger, Kulkami, & Mashelkar, 1992; Cussler, Stokar, & Varberg, 1984; Prazeres, 1995; Vasheghani-Farahani, Cooper, Vera, & Weber, 1992). Although such polymers were considered to be an attractive alternative for the concentration of proteins, no progress has been made recently.

In this paper, we report the synthesis of superabsorbent polymers in aqueous solutions by a graft copolymerization method using potato starch as a basic macromolecular material, acrylic acid as a monomer, ammonium persulfate as an initiator and glycerol as a crosslinker. The effect of the ratio of these components on the performance of the polymer in concentrating extraction solutions was investigated. By adjusting the amount of crosslinker, an optimal polymer was synthesized that can be used for the simple and fast concentration of extraction solutions at room temperatures or low temperatures, with high recovery rates of target substances.

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2. Materials and methods

2.1. Materials and reagents

Cordyceps militaris was cultured in the *Cordyceps* culture center of our laboratory, with the extraction solution prepared according to our previous report (Ni, Zhou, Li, & Huang, 2009). Sweet potato, sweet potato starch and potato starch were purchased from local markets.

HPLC-grade solvents were purchased from Burdick & Jackson Inc. (Muskegon, MI, USA). All other reagents were of analytical or biochemical grade and were purchased from local suppliers.

2.2. Preparation of absorbent polymers

The preparation of absorbent polymers was based on a copolymerization method in water solution (Durmaz & Okay, 2000). The synthesis of polymers consisted of three reactions: (1) starch powder was suspended in distilled water in a 200-ml beaker and gelatinized with NaOH for 30 min with stirring, (2) acrylic acid was neutralized with NaOH in a 100-ml beaker and mixed thoroughly and (3) neutralized acrylic acid was added to the gelatinized starch solution in a water bath at the appropriate temperature, mixed and copolymerized by sequentially adding $K_2S_2O_8$ as a chemical initiator and glycerol as a cross-linker. The reaction was complete after mixing for 10 min. The polymer was washed with distilled water to remove unpolymerized substances, dried in a 70 °C oven and smashed to a particle size of 0.35 mm for use.

2.3. Determination of water uptake and gel strength of the polymers

Smashed resins were weighed in a 250-ml beaker, and a sufficient volume of distilled water or solution was added. After 24 h at room temperature, the fully absorbed polymer was filtered through pre-weighed wet filter paper and weighed after water or solutions were filtered. The water uptake was calculated as absorbed water per gram of polymer.

Gel strength was defined as the minimum force required to break the fully imbibed gel. A block of fully absorbed polymer with smooth surfaces was cut and put into a beaker on the right tray of a balance. A plunger with a smooth end surface of 1 cm^2 was vertically fixed with its bottom surface touching the gel surface. On the left tray of the balance, water was gradually added to lift the right tray of the balance until the gel was broken by the plunger. Gel strength (kPa) was calculated as the amount of water added over the surface area of the plunger.

2.4. Concentration of solutions with concentrating polymers

Two methods were tested for the concentration of solutions with synthesized polymers. The first involved adding the polymers directly into the solutions. The second method involved adding polymers wrapped by a membrane with a molecular cutoff of 500 Da (membrane 500) or 100 Da (membrane 100) into solutions. The polymer was soaked completely in the solution and allowed to be fully absorbed in the solution. After filtration or centrifugation, the volume of concentrated solutions was measured. The amount and recovery rate of each target substance in the concentrated solutions were determined.

The membranes used for wrapping the polymers, purchased from Shanghai Green Bird Science and Technology Co. (Shanghai, China), were CE Precision Dialysis Bags with a flat width of 31 mm and a molecular-weight cutoff of 500 Da (MWCO 500) or 100 Da (MWCO 100). One gram of polymer was added to a 25-cm-long membrane bag with one end tied, and 2 ml of distilled water was injected into the bag to moisturize the polymers and increase the adsorption speed. For concentration treatments, the membrane bag with the polymer remained in the solution with shaking at 100 rpm for 12 h. After concentration, the bag was washed with 1/10 volume of distilled water. The concentrated solution and washing solution were combined together, and target substances were analyzed.

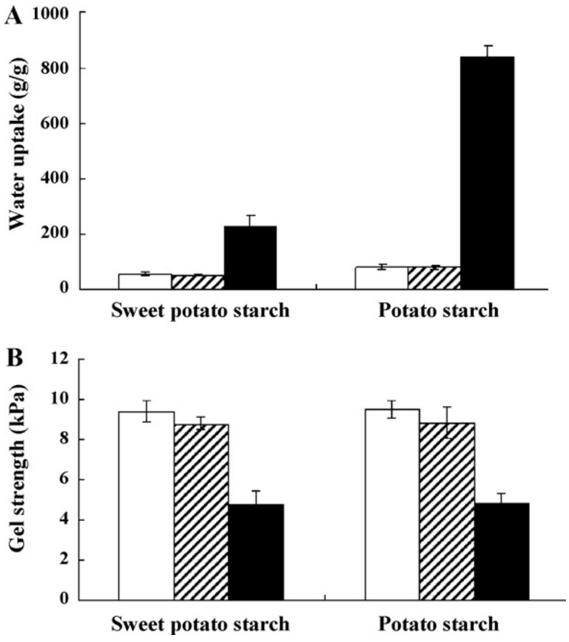

Fig. 1. Water absorbency (A) and gel strength (B) of the polymers made from sweet potato starch and potato starch. (□) 0.9% NaCl solution, (▨) *Cordyceps* extraction solution, (■) distilled water.

2.5. Content determination of protein, polysaccharides, cordycepin and tyrosine

Protein content was determined by the Coomassie Brilliant Blue G-250 method with bovine serum albumin (BSA) as a standard. Proteins were analyzed by SDS-PAGE with a 3% stacking gel and a 10% separating gel, as in our previous report (Wu, Pan, Yu, & Li, 2010). Polysaccharide content was determined by the phenol sulfuric acid method using sucrose as a standard. Cordycepin content was determined by HPLC with a YMC-packed ODS column (4.6 mm × 250 mm, 5 μm), using 20% methanol as the mobile phase with detection at 260 nm, as in our previous report (H. Ni et al., 2009). Tyrosine was analyzed using the same conditions as those used for cordycepin, except that water was used as the mobile phase and detection occurred at 195 nm.

All experiments were repeated at least three times, and the data presented are the means of three independent replicates.

3. Results and discussion

3.1. Preparation of superabsorbent polymers for maximum water uptake

The synthetic process of creating superabsorbent polymers by a graft copolymerization method in aqueous solution (Durmaz & Okay, 2000) was divided into three reactions: starch gelatinization with NaOH in water, neutralization of acrylic acid with NaOH and the copolymerization of starch and acrylic acid with $K_2S_2O_8$ as a chemical initiator and glycerol as a crosslinker. The effects of varying the ratio of reagents and reaction conditions on water uptake and gel strength of the synthesized polymers were studied. Optimal reagent combinations and reaction conditions were determined.

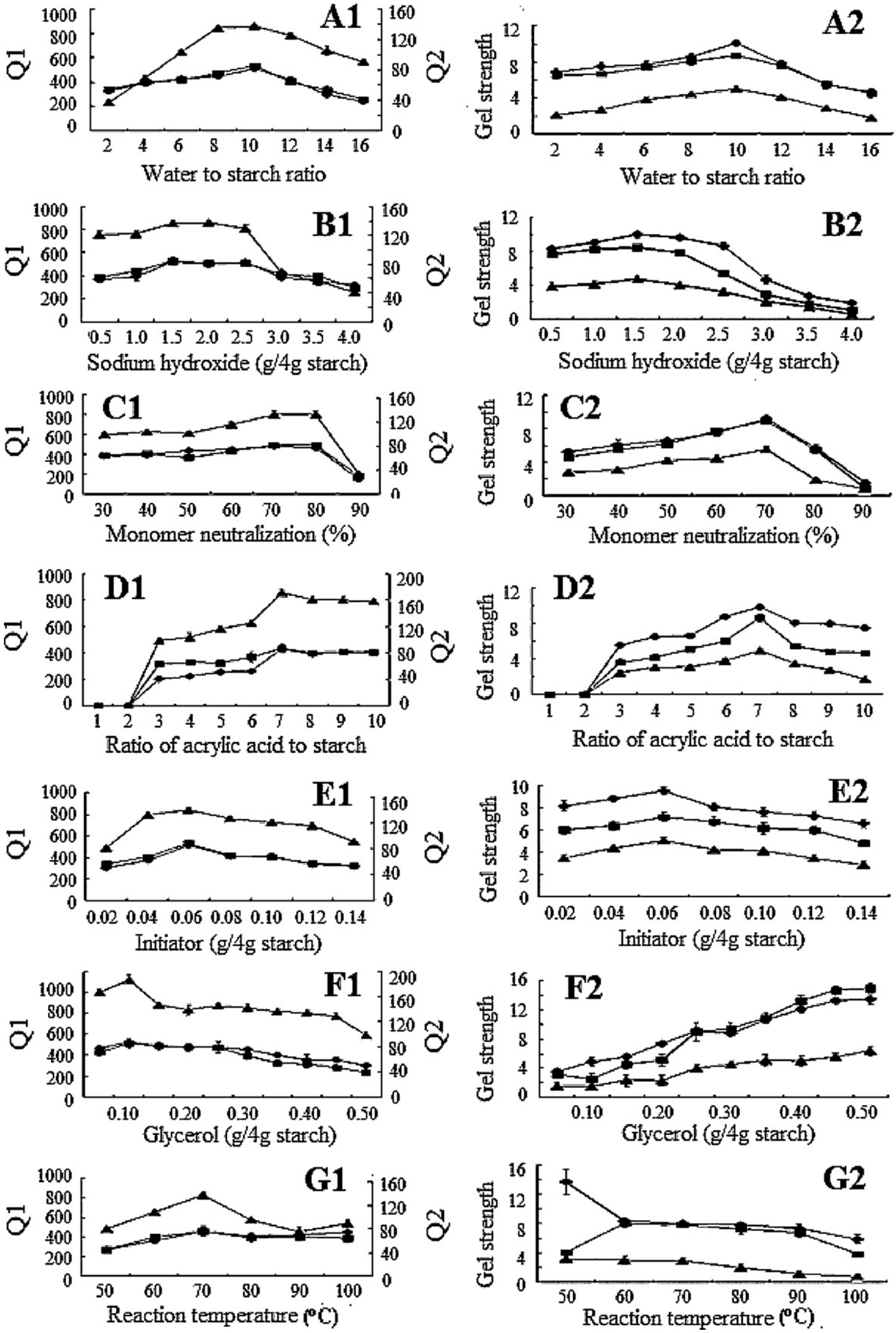

Fig. 2. Effects of reaction reagents and conditions on water uptake and gel strength of synthesized polymers. Q1: water absorbency in distilled water, Q2: water absorbency in solution. (▲) Distilled water, (♦) 0.9% NaCl solution, and (■) *Cordyceps* extraction solution.

Two types of starch were tested as basic macromolecular substrates for the synthesis of polymers. As shown in Fig. 1A, polymers made from potato starch absorbed 838 times their own weight in water, a level that was much higher than that reached by the polymers made from sweet potato starch, which absorbed 230 times their own weight in water. Polymers made from both kinds of starch showed similar gel strength (Fig. 1B). Potato starch was selected for the synthesis of superabsorbent polymers.

For starch gelatinization with NaOH, the starch content in water and the amount of NaOH added were optimized regarding the performance of the synthesized polymers. As shown in Fig. 2A1 and A2, water-to-starch ratios from 2 to 16 were tested. Both the water uptake and gel strength of the polymers increased with water-to-starch ratios between 2 and 10, reached a peak at a ratio of 10 and then decreased from a ratio of 10 to a ratio of 16. A water-to-starch ratio of 10 was determined to be optimal for starch gelatinization. Amounts of NaOH ranging from 0.5 g to 4.0 g were tested for gelatinizing 4 g of starch. The polymer showed highest water uptake and gel strength with 1.5 g of NaOH (Fig. 2B1 and B2). The optimal starch gelatinization can thus be obtained with 1.5 g NaOH for 4 g of starch in 40 ml of water.

The effect of acrylic acid neutralization ranging from 30% to 90% by NaOH on the performance of synthesized polymers was tested. As shown in Fig. 2C1 and C2, the synthesized polymer showed the highest water uptake and gel strength at a neutralization level of 70%.

Ratios of acrylic acid to starch from 1.0 to 10.0 were tested, and the performance of the synthesized polymers was analyzed. Both water uptake capacity and gel strength of the resins increased as the ratio of acrylic acid to starch increased from 1.0 to 7.0, and these parameters decreased as the ratio increased between 7.0 and 10.0. The optimal ratio of acrylic acid to starch was determined to be 7.0 (Fig. 2D1 and D2). The addition of 0.6 g $K_2S_2O_8$ to a reaction with 4 g of starch was determined to be the optimal amount of initiator, and the polymer synthesized in that reaction showed the highest water uptake and gel strength (Fig. 2E1 and E2).

Using a reaction containing 4 g of starch, glycerol was tested as a crosslinker at amounts ranging from 0.05 g to 0.5 g, and the performance of synthesized polymers was analyzed. Water uptake increased when glycerol content increased from 0.05 to 0.1 g and then decreased for amounts ranging from 0.10 to 0.50 g, with the highest water uptake at 0.10 g of glycerol (Fig. 2F1). However, gel strength increased as the amount of glycerol increased from 0.05 to 0.5 g (Fig. 2F2). Polymerization reaction temperatures ranging from 50 °C to 100 °C were tested, and a temperature of 70 °C was determined to be optimal (Fig. 2G1 and G2).

Based on the results described above, a superabsorbent polymer with maximal water uptake can be synthesized with the following composite combinations and reaction conditions: gelatinization of 4 g potato starch with 1.5 g NaOH in 40 ml of water, neutralization of acrylic acid to 70% with NaOH and polymerization of 4 g starch at 70 °C with 28 g of acrylic acid, with 0.05 g of $K_2S_2O_8$ as an initiator and 0.10 g of glycerol as a crosslinker. The synthesized polymer can absorb 1180 ± 42.9, 86.3 ± 3.2, 223.7 ± 21.8, 85.9 ± 4.9 and 82.6 ± 6.5 times its own weight of distilled water, 0.9% NaCl, BSA (100 μg/ml), *Cordyceps* extract and sweet potato extracts, respectively.

3.2. Preparation of concentration polymers by crosslinking modifications

The superabsorbent polymer synthesized above was tested for protein solution concentration. There was approximately 20% protein loss after the solutions were concentrated to 1/3 of their original volumes. With the goal of synthesizing a polymer to be used specifically for concentrating solutions with an improved rate of recovery of target substances, a series of polymers were synthesized with different amounts of glycerol as a crosslinker, and their concentration efficiencies were investigated.

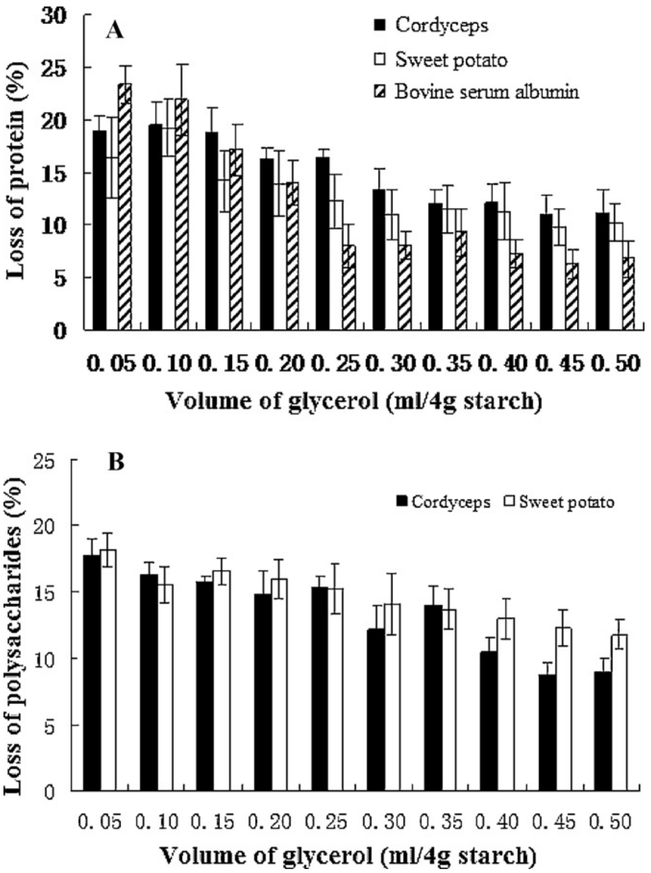

Fig. 3. Effects of glycerol amount used in the synthesis of polymers on the rate of protein and polysaccharide loss in two solutions.

As shown in Fig. 3A, the loss of protein content in solutions of BSA, *Cordyceps* extract and sweet potato extract decreased with an increasing glycerol amount in the range of 0.05–0.45 g. The lowest level of protein loss was reached at 0.45 g of glycerol, and protein loss remained near this level at glycerol amounts higher than 0.45 g. Similar results were obtained for the concentration of polysaccharide solutions from *Cordyceps* and from sweet potato (Fig. 3B). The loss of proteins and polysaccharides decreased to 10% or less. In other words, recovery rates of 90% or more for both proteins and polysaccharides can be obtained when the solutions are concentrated to 1/3 of their original volume using a synthesized polymer with 0.45 g of glycerol. The polymer can absorb 767.3 ± 32.9, 59.0 ± 4.1, 211.7 ± 17.8, 47.0 ± 3.4 and 49.6 ± 4.5 times its own weight in distilled water, 0.9% NaCl, BSA (100 μg/ml), *Cordyceps* extract and sweet potato extract, respectively. The water adsorption capacities of the polymer increased with the dilution level of the *Cordyceps* extraction solution (Fig. 4).

The results indicate that increasing the crosslinking of the polymer by adding more glycerol during the polymerization process can improve the performance of the polymer in concentrating solutions and by decreasing the loss of proteins and polysaccharides. Similar to the results found by Prazeres (1995) using the superabsorbent polymer Sanwet IM-5000-SG from Hoechst for the concentration of dilute solutions of BSA, a loss of proteins and polysaccharides of approximately 10% was detected in our experiments. This loss might be due to sedimentation of the macromolecules and/or irreversible adsorption to the polymer surface during concentration.

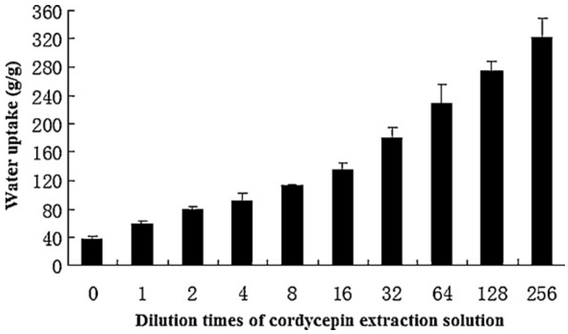

Fig. 4. Effect of dilution of *Cordyceps* extraction solution on the water uptake of the concentrating polymer.

Table 1
Recovery rates (%) of proteins and polysaccharides after concentrating to 1/3 of the original volume with the concentrating polymer.

	Bovine serum albumin	*Cordyceps* extracts	Sweet potato extracts
Protein			
Direct concentration	94.6 ± 1.8	91.5 ± 2.2	90.5 ± 3.0
Wrapped with membrane[a]	95.2 ± 0.8	94.9 ± 0.7	90.9 ± 5.4
Polysaccharides			
Direct concentration	–	92.2 ± 3.9	91.8 ± 5.6
Wrapped with membrane[a]	–	92.7 ± 3.3	90.9 ± 3.1

[a] The polymer was wrapped by a membrane with a molecular cutoff of 500 Da.

3.3. Concentration of macromolecular solutions with concentration polymers

Protein and polysaccharide solutions were concentrated to 1/3 of their original volumes by the concentration polymers using two methods: addition of the polymer directly into solution or addition of the polymer wrapped with a membrane (MWCO 500) into solutions. As shown in Table 1 and Fig. 5, the recovery rates of proteins and polysaccharides were between 90.5% and 95.2% in three protein solutions consisting of BSA, *Cordyceps* extract and sweet potato extract and in two polysaccharide solutions consisting of *Cordyceps* extract and sweet potato extract. The recovery rates of proteins and polysaccharides were not significantly different between the directly concentrated samples and the samples concentrated with membrane-wrapped polymers. These results indicate that macromolecular solutions can be effectively concentrated by directly adding the polymers into solutions.

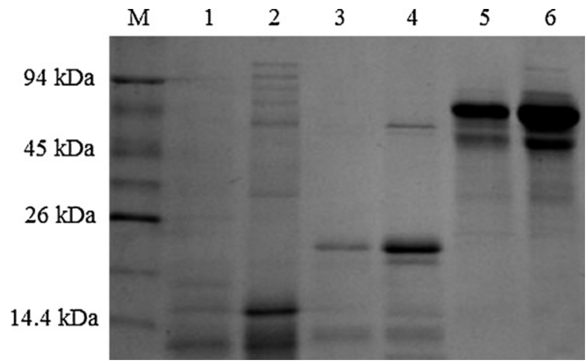

Fig. 5. Protein profiles in several solutions before and after concentration with the concentrating polymer. M, molecular marker; protein profiles before (1) and after (2) concentration of *Cordyceps* extracts, before (3) and after (4) concentration of sweet potato extracts and before (5) and after (6) concentration of bovine serine albumin.

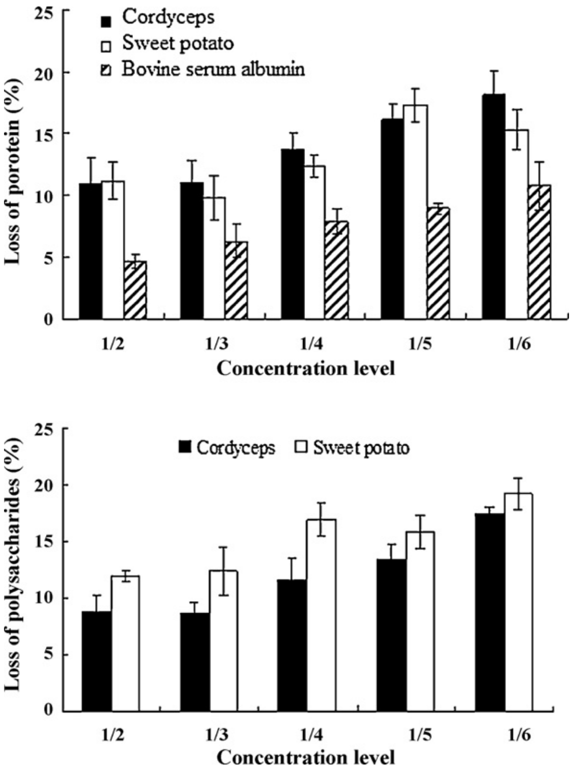

Fig. 6. Effect of concentration level on the loss of proteins and polysaccharides after concentration with the concentrating polymer.

Solutions of proteins and polysaccharides were concentrated to different levels ranging from 1/2 to 1/6 their original volume, and their recovery efficiencies were assessed. Protein and polysaccharide levels decreased by 5–12% when solutions were concentrated to 1/2 or 1/3 of their original volumes. However, losses increased with higher levels of concentration, with a 10–20% loss when solutions were concentrated to 1/6 of their original volumes (Fig. 6). The optimal level of concentration by the polymer was found to occur at 1/3 of the original volume.

3.4. Concentration of small-molecule solutions with concentration resins

The concentration of small-molecule solutions was tested using the modified concentrating polymer. Three different methods were tested for the concentration of cordycepin and tyrosine solutions. The polymer was added directly into the solution, or it was wrapped by a membrane with a cutoff of 500 Da or a membrane with a cutoff of 100 Da before being added into the solution. The recovery rates of cordycepin and tyrosine were measured after the solutions were concentrated to 1/3 of their original volumes.

As shown in Table 2, the recovery rates of cordycepin and tyrosine, both in pure solutions and in crude extracts, ranged from 49.5% to 53.2% when solutions were treated with the polymer directly

Table 2
Recovery rates (%) of small-molecule substances after concentrating to 1/3 of the original volume with the concentrating polymer.

	Cordycepin standard	*Cordyceps* extracts	Tyrosine	Tyrosine standard extracts
Direct concentration	49.7 ± 1.7	52.7 ± 3.8	53.2 ± 3.5	49.5 ± 3.1
Wrapped with MWCO 500	52.0 ± 4.8	68.7 ± 2.1	59.6 ± 3.5	54.9 ± 5.6
Wrapped with MWCO 100	91.1 ± 2.4	90.0 ± 2.9	89.8 ± 2.8	89.0 ± 3.8

and from 52% to 68.7% when solutions were treated with the polymer wrapped with the 500-MWCO membrane. When the solutions were treated with the polymer wrapped with the 100-MWCO membrane, the recovery rates of both cordycepin and tyrosine were between 89.0% and 91.1%. These results indicate that the concentration of small-molecule solutions with polymer alone or with the polymer wrapped in 500-MWCO membrane involves high losses and low recovery rates of the target substances. However, when these solutions were concentrated with the polymer wrapped in the 100-MWCO membrane, both cordycepin and tyrosine showed high recovery rates of about 90%, which is an excellent recovery rate for the concentration of small-molecule solutions.

4. Conclusions

This study assessed the synthesis of starch-based superabsorbent polymers and the effects of altering the proportion of synthetic materials in the synthesized polymers on their performance in concentrating extraction solutions. A polymer with maximal water uptake was synthesized, with a composition of potato starch:water:NaOH:acrylic acid (at 70% neutralization):$K_2S_2O_8$:glycerol at a ratio of 4:40:1.5:28:0.05:0.10. This substance can absorb 1180 times its own weight in distilled water, 223.7 times its weight in BSA solution (100 μg/ml) and 82.6–86.3 times its weight in 0.9% NaCl or extraction solutions. By adjusting the amount of crosslinker, an optimal polymer for concentrating extraction solutions (i.e., a concentrating polymer) was synthesized, with a composition of potato starch:water:NaOH:acrylic acid (at 70% neutralization):$K_2S_2O_8$:glycerol at a ratio of 4:40:1.5:28:0.05:0.45. This substance can absorb 767.3 times its own weight in distilled water, 211.7 times its weight in BSA solution (100 μg/ml) and 47.0–59.0 times its weight in 0.9% NaCl or extraction solutions. The concentrating resin can be used directly to concentrate macromolecular solutions or can be wrapped in MWCO 100 and then used for concentrating small-molecule solutions, with a 90% or higher recovery rate. Results demonstrated that superabsorbent polymers with the appropriate level of crosslinking can be used for the simple and efficient concentration of extraction solutions at room or low temperatures at low cost and low energy consumption. The method is particularly useful for concentrating solutions of enzymes and other temperature-sensitive substances.

Acknowledgments

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