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Concentration of Whey Proteins by Using Temperature-Sensitive Polymer Gel Poly(N-isopropylacrylamide)

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Summary: In this work, the use of a temperature-sensitive polymer gel, poly(N-isopropylacrylamide), for the concentration of whey proteins was studied. The studied variables were: gel mass/solution volume ratio and concentration temperature. The concentration percentage and the selectivity were determined. The gel 20 × 5 (20% w/w total monomer/solution and 5% w/w crosslinking agent/total monomer), contacted with whey proteins solutions, at 5 °C and at 20 °C, was capable of concentrating the solution, in protein, from 10 to 33%, depending on the gel mass/solution volume ratio. The separation efficiencies, for the different studied systems, varied from around 40 to 80%. The results were discussed in the context of gels thermodynamics and through correlations between synthesis parameters and structure of the obtained gels. The obtained results for the concentration of whey proteins solutions, by using temperature-sensitive polymer gel, poly(N-isopropylacrylamide), showed that the Gel Process can indeed be used as an advantageous alternative for such separation, either from an economic or from an environmental view point.

Keywords: concentration process; milk serum; Poly(N-isopropylacrylamide); temperature-sensitive gels; whey proteins

Introduction

Milk serum is an important byproduct of the dairy industry, considering the produced volume and its nutritional composition. However, around half of the produced whey is eliminated as an effluent or is used as soil fertilizer, resulting in alimentary energy loss as well as economic loss. When disposed in the environment, without any treatment, the serum constitutes the principal pollutant source of the environment generated by the dairy industry, considering that its pollutant power is about ten times the domestic sewage one. The other half of the serum produced in the world is processed into several food and pharmaceutical products. Usually, 10 L of milk produces 1 kg of cheese and 9 L of milk serum.[1,2]

Some of the most valuable components of milk serum are the proteins, also known as ''whey proteins'', for their excellent nutritional, technological and functional properties. About 96% of milk proteins remain in the milk serum. Nevertheless, their concentration in this liquid is low, ranging from 0.7 to 1.2%, being necessary the use of separation operations for concentrating them.[3]

The main globule proteins present in the milk serum are β-lactoglobulin (β-Lg), α-lactoalbumin (α-La), bovine serum albumin (BSA), immunoglobulins (Ig), lactoferrin, lactoperoxidase, glicomacropeptides

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and proteosis-peptones. The molecular mass of these proteins range from 4.1 to160.0 kDa.[4,5]

The processes, usually used for concentrating milk serum proteins, like ultrafiltration, chromatography, crystallization and thermal denaturation are expensive, time-consuming and limited to small scale use. So, the development of a methodology for using a concentration process that is fast, economic and that can be used on large scale operation is extremely important.[2]

The concentration process, used in the present work, was developed by Freitas and Cussler.[6,7] This process involves the use of crosslinked polymer gels, whose swelling is a dramatic function of temperature, as extraction solvents. The gels can be used to concentrate dilute solutions of macromolecules because they absorb water and other low molecular weight species, but do not absorb high molecular weight solutes; and they can be easily regenerated because their volume is a strong function of temperature: a slight increase in temperature collapse the gel, releasing the absorbed species.

Polymer gels have been widely used in many different applications, including concentration of macromolecular solutions, column packing materials for chromatography, drug delivery systems and cell culture substrata.[6–16] One of these gels, poly(N-isopropylacrylamide) (PNIPAAm), swells to a large extent in water, at a low temperature, and shrinks as the temperature is increased, showing a first-order phase transition around 34 °C, behaving as a polymer solution with a lower critical solution temperature (LCST).[6–8,17–21]

In this paper, we report the use of a temperature-sensitive polymer gel, poly(N-isopropylacrylamide), for the concentration of whey proteins. A gel with an specific monomer/crosslinking agent ratio was used for such concentration. Different gel mass/solution volume ratios and different concentration temperatures were used. The concentration percentage and the selectivity were determined.

Experimental Part

The sweet skim powdered milk serum, from cheese fabrication, used in this work, was gently supplied by Sooro (PR-Brazil). The milk serum solution was prepared by dissolving 0.5 g of powdered milk serum in 50 mL of milli-Q water.

Poly(N-isopropylacrylamide) gel was synthesized by free-radical solution copolymerization. The monomer N-isopropylacrylamide (Sigma-Aldrich) was purified and recrystalized to yield 100% NIPAAm, as determined by HPLC. The crosslinker, N,N'-methylenebisacrylamide (Sigma-Aldrich) was electrophoresis grade and the initiators, ammonium persulphate (Vetec) and sodium metabisulphite (Sigma-Aldrich) were reagent grade; they were all used as received. The polymerization was carried out under a nitrogen atmosphere, at 10 °C for 24 h, in cylindrical glass tubes. The gel composition used in this study (20×5 gel) was 20 g of total monomers (N-isopropylacrylamide plus N,N'-methylenebisacrylamide) per 100 g of milli-Q water, with 5 g of crosslinker (N,N'-methylenebisacrylamide) per 100 g of NIPAAm plus crosslinker. That is, 20% w/w total monomer/solution and 5% w/w crosslinking agent/total monomer. Details of the monomer purification and gel synthesis are reported elsewhere.[22]

After polymerization, the gel was thoroughly washed with milli-Q water to remove any residue of unreacted monomer(s) and then cut into small pieces with a size ranging from 48 to 74 mesh. The particles were dried at 60 °C for 24 h.

For the gel mass/milk serum solution ratio study, a mass of 0.5 g of the 20×5 gel was added to 50 mL of milk serum solution (feed), whose concentration in total protein was determined, in triplicate, by a modified Lowry's method (Lowry-BCA). This system was kept at 5 °C for 24 h. The swollen gel was, then, separated from the solution. This concentrated solution (raffinate) was submitted to protein determination, in triplicate, by using the same method (Lowry-BCA). This procedure was repeated by using

different gel masses (1.0; 1.5 and 2.0 g) for 50 mL of milk serum solution. The swollen gel, containing water and some other low molecular weight species, was collapsed at 60 °C, separated from the desorbed solution (water plus some low molecular weight species), thoroughly washed with milli-Q water and dried at 60 °C for 24 h. This dried gel was ready to be used in another concentration cycle, if desired.

For the temperature effect study, the above procedure was repeated, the system (dry gel and milk serum solution) being kept at 20 °C for 24 h.

Results and Discussion

The average total protein concentration, obtained through modified Lowry's method determination, for the feed solution (0.5 g of powdered milk serum in 50 mL of milli-Q water), was 1.7122 g/L, with a standard deviation of 0.0948. This average value of concentration was used in all experiments in order to compare with the concentration of the raffinate. The results for different gel masses and different temperatures are shown in Figure 1.

The obtained results demonstrate that the 20×5 gel is selective towards the proteins, considering that there is an increase in protein concentration at both temperatures and for all gel mass/solution volume ratios. For both temperatures (5 and 20 °C), the higher the gel mass, the higher the % concentration increase. Considering, therefore, that the gel is selective towards the proteins, the higher concentration obtained for higher gel mass/solution volume ratios is justified as for a certain mass degree of swelling, a higher gel mass absorbs a higher solution quantity. As there is a partition, *i.e.*, the gel is selective, it means that a higher quantity of solution absorbed, composed mainly by low molecular weight species, implies in a higher concentration increase, in proteins, in the non-absorbed solution.

For determining the selectivity of the process concentration for each system via temperature-sensitive gel, that is, for measuring the degree of exclusion of proteins at different conditions, the separation efficiencies were calculated. The separation efficiency, η, is defined as the actual concentration difference, ΔC, between the initial solution (feed) and the final solution (raffinate) divided by the maximum concentration difference, ΔC_{max}, which would be attained if all the solute (proteins, in this case) of the initial solution were recovered in the raffinate. Physically, it can be understood as a measure of the degree to which a solute is excluded from the gel as water or solvent is absorbed. Thus, an efficiency of 100% means that the gel absorbs no solute, *i.e.*, the solute is completely excluded from the gel. However, if the solute is absorbed by the gel, along with the solvent, such that the raffinate concentration is equal to the feed

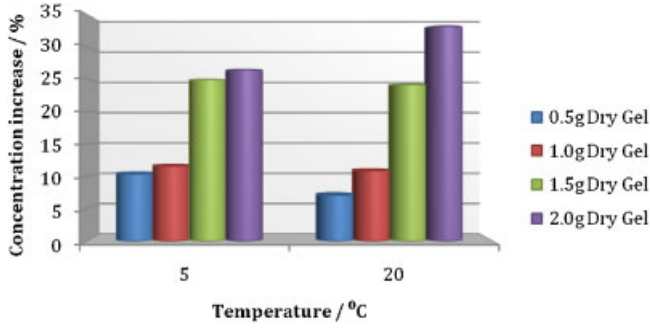

Figure 1.
Influence of gel mass and concentration temperature on the protein concentration.

concentration, the separation efficiency is 0%. This quantity is given by Equation 1:

$$\eta = (\Delta C/\Delta C_{max}) \text{ x } 100 = [(C_f/C_i) - 1]/[(Vi/Vf) - 1] \quad (1)$$

where C_i and C_f are the concentrations and V_i and V_f are the volumes of the feed and the raffinate, respectively.

The obtained separation efficiencies varied between 39.73%, for the system 2.0 g of gel, at 5 °C, and 81.75%, for the system 0.5 g of gel, at 20 °C, with an average equals to 59.42%, as shown in Figure 2.

Higher values of separation efficiencies, 76.22% at 5 °C and 81.75% at 20 °C, were found for the systems with a lower gel mass, 0.5 g.

The obtained results concerning the selectivity, demonstrates that the 20×5 PNIPAAm gel excludes, selectively high molecular weight species, whey proteins in this case, absorbing preferentially the low molecular weight species. As this process can be conducted at low temperatures, below the gel transition temperature (≈34 °C), in the present study at 5 °C and at 20 °C, and this exclusion concentrates the solution gently, there will be no loss of protein activity, a fundamental aspect concerning any separation process applied to the biological area.

Entrainment of small amounts of raffinate between the gel particles can compromise the separation efficiencies. The observed significant variation of the obtained separation efficiencies, for the studied systems, is probably due to the entrainment of proteins on the surface of the gel particles. That is, proteins which are effectively excluded by the gel, but are not determined in the concentrated solution because, despite of not being absorbed by the gel, were adhered to its surface. The fact that the higher separation efficiencies were found when the lower masses of gel (0.5 g) were used corroborates this observation. Lower masses means less gel particles, therefore, lower superficial area for entrainment. Nevertheless, there is no linear relationship between entrainment effect and gel masses, as seen from the results for other gel masses used (1.0 g; 1.5 g and 2.0 g). They suggest the need for further investigation of this entrainment effect.

The obtained results for the different studied temperatures allow one to register that there was no significant change on the average separation efficiency as a function of temperature. This result can be explained by the fact that temperature influences, basically, the phase behavior of the gel, that is, the higher the temperature, the lower the degree of swelling.[6,7]

Considering the use of this process in large scale, the fact that selectivity is not a strong function of temperature besides the strong dependence of the degree of swelling on temperature, as reported elsewhere,[6,7] points out for the conduction of the concentration step at lower temperatures, usually more adequate to biological systems. At such temperatures, as there is a larger gel expansion with no significant change on the selectivity, it's possible to

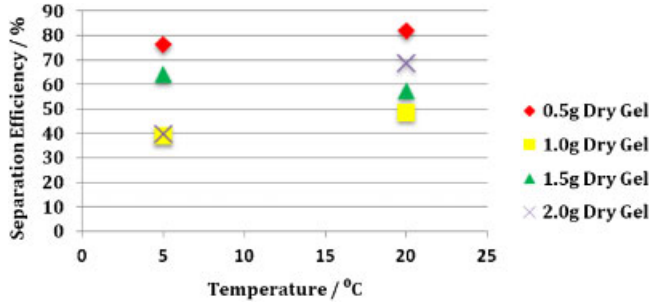

Figure 2.
Influence of gel mass and concentration temperature on separation efficiencies.

achieve a higher concentration of the feed solution.

Conclusion

The 20×5 poly(N-isopropylacrylamide) gel, when contacted with milk serum solution, at low temperatures, below its phase transition, swells excluding the whey proteins. So, this gel is selective to milk serum proteins and can be used as an extraction solvent for the concentration of such system. Due to its thermodynamic behavior, this gel, after being separated from the concentrated milk serum solution, can be regenerated with an increase in temperature. In the present work, the gel was collapsed at $60\,^{\circ}C$, releasing the absorbed low molecular weight species, and could be used again in another concentration cycle.

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