# Concentrating Cellulase Enzymes using a Temperature-Sensitive Hydrogel.

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## 1. Abstract

The swelling behaviour of a poly(N-isopropylacrylarnide) temperature sensitive hydrogel in fermentation broth was determined as a function of the temperature and compared with that in water. The feasibility of the gel as a device for separation of cellulase enzymes, produced by the fermentation of the fungus Trichoderma reesei RUT C-30, was also studied. For the same particle size, gels with different composition showed differences in the swelling time and in the extent of swelling. The time required for collapsing was independent of both the monomer concentration and the degree of crosslinking. Upon successive swelling-collapsing cycles, the gel showed consistency in the volume reached; however, such values were smaller than those values obtained during the swelling behavior determination. During swelling, the gels absorb water and low molecular weight solutes, excluding macromolecules such as proteins. The enzymes were concentrated in the solution non- absorbed by the gels. Mass balances for fermented broth, total soluble protein and enzyme activity showed a recovery close to 100%.

## 2. Introduction

Traditional methods for enzyme purification [1-3] are either energy demanding (i.e. ultrafiltration) or harmful to the enzyme (i.e. solvent precipitation). The use of temperature-sensitive hydrogels has been proposed as an alternative method for enzyme purification by Cussler and coworkers [4, 5]. This process is useful to separate bioproducts due mostly to the mild separation conditions and to the reusability of the gel.

The purification method using temperature sensitive hydrogels takes advantage of the change in the gel volume during a phase transition, and is based on the size exclusion

of macromolecules. The size exclusion of solutes, the change in volume and the reversibility of the phase transition of the gel can be used for the concentration of solutions containing macromolecules.

The phase transition of poly(N-isopropylacrylamide) (pNIPA) hydrogels occurs at temperatures as low as 31-33 degC [6]. The phase transition is macroscopically visible as a change in volume of the gel particles. As the gel is warmed, it goes from a swollen to a collapsed state. The volume change is reversible upon lowering the temperature [6]. pNIPA hydrogels have been widely used in the biotechnology field [7], i.e. for soy protein isolation [8], for drug delivery [9, 10], and for enzyme immobilization [11, 12] and purification [13, 14]. These hydrogels separate solutes based on the solute molecular weight and the gel composition. Solutes with molecular weights of 10,000 daltons [15] or sizes of 3 nm [5] have been proposed as the low limit for effective separation. The limit can be changed by altering the gel composition. Gels formed by higher monomer concentration or higher crosslinking degree will exclude smaller molecular weight solutes due to smaller cage sizes.

## 3. Experimetal

*3.1 Gel preparation*

pNIPA hydrogels were produced by a free radical polymerization at 4 degC, as detailed elsewhere [6, 8, 13]. Three levels of crosslinker were used to produce hydrogels with different crosslinking densities refered as low, medium and high. The effect of hydrogel particle size on its swelling/collapsing characteristics was studied using five different particle sizes: small and large extruded hydrogels (1.2 and 1.7 mm die orifices), hydro gel chopped by **a** razor blade, small and large hydrogel cubes (3 and 5mm size)

*3.2 Swelling measurements*

1.0 g of xerogel (dry basis) was placed in a graduated cylinder; hydrogel volume was determined directly in the graduated cylinder. The volumes of hydrogels with various compositions were measured as the temperature was changed from 4 degC to 45 degC. The gel was immersed in 55 mL of fermented broth. Water was also used as a comparison.

*3.3 Enzyme purification*

The enzyme solution of 1.8 filter paper units (FPU) and pH 6.3 was prepared using

commercial cellulase enzyme from ***Trichoderma reesei*** ATCC 26921 (Sigma). 1.0 g of xerogel and a volume of cellulase enzyme solution were retained in a Plexiglas cylinder. The gel volume was measured prior to the raffinate and permeate recovery. The raffinate and the permeate were collected in graduated cylinders and their volumes collected were then measured.

## 4. Results and Discussion

Figure 1 shows changes in volume for pNIPA hydrogels as function of time and temperature. For the same particle size, gels with different composition showed differences in the swelling time and in the extent of swelling. The time required for collapsing was independent of both the monomer concentration and the degree of crosslinking. Upon successive swelling-collapsing cycles, the gel showed consistency in the volume reached; however, such values were smaller than those values obtained during the swelling behavior determination.

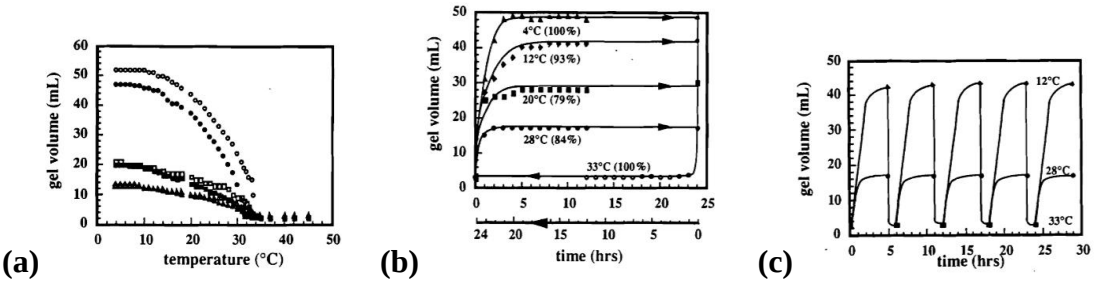


***Figure 1***. *p(NIPA) hydrogel (HG) volume change as a function of time and temperature for several crosslinking densities: low (L), medium (M) and high (H):* ***(a)*** *O , L-HG in water; λ , l-HG in fermented broth; □ M-HG in water; ν, M-HG in fermented broth; Δ , H-HG in water; σ , H-HG in fermented broth. HG volume in fermentation broh is the average of four determinations; the HG volume in water was done once.* ***(b)*** *Volume change of L-HG with time during long (24 hr) temperature shifts between 33 degC (o ) and temperature below the collapse temperature: σ ,4 degC; υ , 12 degC; ν ,20 degC; ,28"C. The numbers in parenthesis indicate the percentage of final volume (volume at 24 hrs) with respect to the volume of the HG as a function of the temperature shown in Fig.5.* ***(c)*** *Volume change of L-HG during short time (5 hr) from 33 degC ( ν ) to 12 degC ( υ ) or 28 degC ( λ ) five successive times.*

A concentrating factor (CP) was defined for the hydrogels. In this case, the CP is defined as the ratio between the mass of the solute in the raffinate and that in the feed. Such a definition will allow one to know how much solute is being recovered with respect to the amount fed in every concentration step. The results are shown in Table 2. For the

total soluble protein, the values were comparable. The protein and the enzyme activity CP for the low crosslimking density hydrogel were found to be statistically significant different (t- test) than the same factors for the others hydrogels. Considering only on the CP, the most attractive hydrogel would be the one that concentrates the largest amount of broth per unit of time. This concept deviates from the usual definition of efficiency [15] where the most efficient is the one closest to ideality. By observing the values in Table 2 and considering the amount of time involved in the separation process for each hydrogel, it can be deduced that the most promising gel would be the high crosslinking density hydrogel.

***Table 1*** *Concentration Factor (CP) for p(NIPA) hydrogels (HG) for several crosslinking densities: low (L), medium (M) and high (H)*

| HG TYPE | No. OF RUNS | PROTEIN CP | ENZYME ACTIVITY CF |
|---|---|---|---|
| | | MEAN ± SD | MEAN ± SD |
| L-HG | 11 | 62.2 ± 3.8 | 61.6 ± 11.5 |
| M-HG | 06 | 73.8 ± 4.8 | 75.8 ± 12.9 |
| H-HG | 06 | 75.7 ± 8.1 | 80.7 ± 15.6 |

## 5. Conslusions

p(NIPA) hydrogels are good candidates as separation materials for concentrating enzyme solutions. The hydrogels show limitations in obtaining a desired concentration in a single step process. However, re-circulation of the permeate stream and multiple step preparations are options to obtain any concentration desired.

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