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FEASIBILITY STUDIES FOR SEPARATION PROCESSES USING ENVIRONMENTALLY SENSITIVE HYDROGELS

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ABSTRACT

Temperature- and pH-sensitive hydrogels can be used to separate or concentrate proteins from dilute solution. Two possible separation processes are discussed here. Experimental partitioning data are used to compare the efficiencies of neutral, weakly acidic, weakly basic, and polyampholytic poly-N-isopropylacrylamide copolymer gels for separating cytochrome c from ovalbumin. For each process, attention is given to the influence of the solute partition coefficient and swelling equilibria on process efficiency.

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I. INTRODUCTION

The swelling properties of temperature-sensitive gels have led to proposals for gel-based separation processes (1-5). Temperature-sensitive hydrogels shrink, sometimes by an order of magnitude, at temperatures often not far removed from ambient. Swelling equilibria and transition temperatures of these temperature-sensitive gels can be altered by incorporation of suitable comonomers. Alternatively, the swelling of a gel can be made sensitive to pH by incorporating a weakly ionizable monomer such as acrylic acid or dimethylaminoethylmethacrylate into the gel structure.

The best-known temperature-sensitive gel is the poly-N-isopropylacrylamide (poly-NIPA) hydrogel. Both Cussler and co-workers and Prausnitz, Blanch and co-workers have proposed processes which use poly-NIPA-copolymer hydrogels for concentrating or separating dilute aqueous protein solutions. Cussler and co-workers [Process A] have proposed the process illustrated in Figure 1a; the solute to be recovered is concentrated in the raffinate phase (1). The feed solution is brought into contact with deswollen gel which swells in the feed solution, absorbing water and low-molecular-weight solutes. The swollen gel is separated from the raffinate and collapsed by increasing the temperature. As the gel collapses, it expels imbibed water and solutes. The deswollen gel is then recycled and the process repeated.

Prausnitz, Blanch and co-workers [Process B] have proposed an extraction process illustrated in Figure 1b; the solute to be recovered is concentrated in the gel, and the gel is deswollen with a temperature and/or pH shift not only for use in another cycle, but also to release the extracted solute (4). This process requires careful consideration of gel chemistry to choose a gel that is selective for one or more solute(s) and, in addition, has the required swelling properties.

Both processes are essentially analogous to a two-phase liquid-liquid extraction process with regeneration of one stream. Temperature-sensitive gels are advantageous because they are "gentle" towards solutes and because a small energy investment can induce a large change in swelling.

In Reference 6, we presented the effects of pH, ionic strength, temperature, and gel charge density on the partition coefficients for selected proteins and other small biomolecules in pH- and temperature-sensitive hydrogels. In this work, we first compare the efficiencies of the processes in Figures 1a and 1b for the separation of cytochrome c from ovalbumin in dilute solution, using experimental data from Reference 6.

We then examine the influence of solute partition coefficient and gel swelling on the efficiencies of both processes, neglecting the relation between gel swelling and partition coefficient. By neglecting this relation, our results are not restricted to a specific gel chemistry. Our goal is to discover how much gel we should use and how large or small a partition coefficient must be to separate and/or concentrate a solute. From these studies, we obtain the ranges of partition coefficient and gel swelling that are most favorable for a specified separation.

II. RESULTS

A. Poly-NIPA-Based Hydrogels as Separation Agents

In Reference 6, we reported that poly-NIPA-based hydrogels are selective for cytochrome over ovalbumin. However, simple partitioning experiments alone do not yield percent of recovery of gel-based separation processes. Recovery and enrichment of the desired solute depend also on the amount of gel per unit of feed.

In Process A (Figure 1a), where the desired solute is to be concentrated in the raffinate, the partition coefficient should be as low as possible. In Process B (Figure 1b), where the

desired solute is to be concentrated in the extract, the partition coefficient should be as high as possible in the swollen state and as low as possible in the collapsed state. In both processes, the major trade-off is between recovery and final concentration of the desired solute.

We now consider the recovery and concentration enrichment for each process using the experimental data of Reference 6 for the partitioning of cytochrome c and ovalbumin in poly-NIPA (I); in poly-NIPA/10% sodium acrylate (SA) (II); in poly-NIPA/10% dimethylaminoethylmethacrylate(DMA), (III); and in poly-NIPA/5% SA/5% DMA (IV). For all gels, the molar percent of crosslinking monomer was 1% on a diluent-free basis and the volume fraction of monomers was 8% in the solution prior to polymerization. In our calculations, we make mass balances around each step of the process.

To characterize the efficiency of the process under specified conditions, we define the recovery or yield of a desired solute as:

$$\frac{\text{mass of desired solute in recovery stream}}{\text{mass of desired solute in feed}}$$

We define the enrichment of the desired solute by the process as:

$$\frac{\text{concentration of desired solute in recovery stream}}{\text{concentration of desired solute in feed}}$$

For each process, Table 1 gives results of our calculations for each of the four poly-NIPA-based hydrogels to separate cytochrome c from ovalbumin. The initial concentration of each protein was 0.05 mg/mL except in experiments with Gel IV, where the initial cytochrome concentration was 0.6 mg/mL and the ovalbumin concentration was 2.6 mg/mL. Process A more effectively concentrates a solute when the ratio of swollen gel to the combined mass of the gel and solution is large. Table 1 presents the maximum enrichment of cytochrome (relative to the

feed solution) that is attained if 90% of the cytochrome is recovered in the raffinate solution. Also shown is the initial mass fraction of dry gel which results in 90% recovery. Only the neutral poly-NIPA gels (Gel I) enrich cytochrome significantly relative to the feed solution. Poly-NIPA gels swell considerably less than the polyelectrolyte gels (Gels II and III), but only slightly less than the polyampholyte gels (Gel IV). Gel I concentrates the protein more than Gel IV because the minimum partition coefficient for protein in Gel I is lower than that in Gel IV. Because the neutral gels swell the least, more Gel I (on a dry-mass basis) is necessary compared to other gels; only three weight percent of dry poly-NIPA in the feed results in 90% recovery of the protein.

For Process B, most solute is recovered when the gel absorbs virtually all the solution. While the process is impractical if all the feed solution is absorbed, this condition represents an upper bound on recovery and enrichment. Table 1 shows that a maximum recovery of 100% can be obtained only by using Gel I because the partition coefficient at 36.4°C is zero. Unfortunately, under these conditions, the protein in the extract is concentrated only 4% more than in the feed solution. The highest enrichment (protein is 33% more concentrated) is obtained by using the weakly basic Gel III, but then maximum recovery falls to 76%. This result is not intuitive because both the protein and the gel are positively charged. Use of the weakly acidic Gel II, into which the protein partitions most favorably, does not enrich the protein.

Results in Table 1 suggest the following conclusions concerning recovery of cytochrome c using various poly-NIPA-based hydrogels: (1) for Process A, the greatest enrichment at 90% yield can be obtained by using poly-NIPA hydrogels because the partition coefficient of cytochrome is lowest at room temperature in poly-NIPA; (2) for Process B, the best combination of enrichment and yield can be obtained by using the poly-NIPA/DMA gels, although the partition coefficient for cytochrome is higher in the weakly acidic and weakly ampholytic gels.

Further, (3), the weakly acidic Gel II cannot be used for simultaneous enrichment (relative to feed concentration) and recovery. Conclusions (2) and (3) are not intuitive. They illustrate that qualitative trends in partition coefficient with gel chemistry do not necessarily correlate with relative efficiencies. A gel which is efficient for concentration enrichment is not always efficient for recovery.

B. Influence of Partition Coefficient and Gel/Solution Ratio on Enrichment and Concentration

We examine now the influence of the solute partition coefficient and gel swelling equilibria on the efficiency of the two proposed processes. To elucidate what ranges of partition coefficient and swelling equilibria are most favorable, we consider hypothetical cases, irrespective of the experimental connection between partitioning and swelling equilibria. Because we do not consider explicitly how we collapse the gels in the processes, these results are relevant to any environmentally responsive gel, not only those sensitive to temperature or pH.

We consider first Process A where the desired solute is concentrated in the raffinate. For this process, the important gel-related variable is the final mass fraction of swollen gel in the raffinate-gel system. (For purposes of comparison, the initial mass of deswollen gel is irrelevant). Figure 2 presents a contour plot of the percentage recovery of solute (defined relative to the amount in the feed) as a function of the hypothetical partition coefficient (from 0-1) and mass fraction of swollen gel. For most values of partition coefficient and mass fraction, recovery is less than 80%. The region of 90-100% recovery is broadest at small partition coefficients and mass fractions, as expected. If the mass fraction of gel is only 1/3, and the partition coefficient is 0.2 or less, the yield is greater than 90%. If, however, the mass fraction of gel is 2/3, a partition coefficient greater than about 0.06 will cause the yield to drop below 90%. Because extremely small partition coefficients are harder to obtain, it is fortunate that the upper bound on partition coefficient (for 90% yield) increases as gel mass fraction decreases. Also, entrainment losses are less significant at lower fractions of gel. Figure 3 presents a contour plot of the concentration of

the solute in the raffinate as compared to that in the feed. For the best case (partition coefficient zero), the solute cannot be concentrated by more than two-fold unless the final mass fraction of gel is above 0.5, and it can never be concentrated above two-fold if the partition coefficient is above approximately 0.45. By comparing the two contour plots, we see that (in general) it is difficult to obtain simultaneously high recovery and high concentration because the peaks of each surface are located in different corners of the partition-coefficient/mass-fraction plane. There is a small region where more than 80% of the solute can be recovered at a concentration 2-3 times that of the feed; that region is bounded by partition coefficients less than 0.2 and mass fractions greater than 0.5.

In Process B, the solute is concentrated in the extract; efficiency depends also on the partition coefficient of the solute in the collapsed gel and the difference in swelling between the swollen and deswollen states. Figure 4 for Process B presents a contour plot similar to that in Figure 2 for Process A. The partition coefficient between the deswollen gel and surrounding gel is taken as zero, which makes the process most efficient. The region of 90-100% recovery is now centered in the opposite corner to that for Process A in the partition coefficient/mass fraction plane (high partition coefficients and high mass fractions). At a mass fraction of 2/3, the partition coefficient must be 2 or greater to achieve more than 80% yield. At a mass fraction of 1/3, the partition coefficient must be close to 10. Partition coefficients above 2 do not seem to be readily attainable for proteins at ionic strengths typical for realistic applications (more than 0.1M); therefore, simultaneous concentration enrichment and high yield is unlikely.

Figure 5 presents a contour plot of the concentration in the extract relative to that in the feed. The surface in this case also is a maximum in the corner opposite to that in Figure 3 (high partition coefficients and low mass fractions). However, the high partition coefficients necessary to obtain greater than three-fold concentration and 80% recovery are generally not obtainable

without the employment of affinity ligands. For extraction (Process B), it appears that less than 2-fold concentration and less than 90% recovery is the norm. If the partition coefficient for the solute in the deswollen gel is greater than zero, the areas of high yield and concentration shrink. Figures 6 and 7 are analogous to Figures 4 and 5, respectively, but the partition coefficient for the solute in the deswollen gel is now 0.5 instead of zero as in Figures 4 and 5. While this is a significant increase in the partition coefficient, the contours are not greatly affected.

We also plotted surfaces for a swelling ratio of twice that of Figures 4 and 5 and found essentially similar contours as those in Figures 6 and 7. The extractive process depends only weakly on partition coefficient (with respect to the deswollen gel) and on the difference in swelling between the swollen and deswollen states. This weak sensitivity probably accounts for the reason why higher efficiencies are attainable for the use of poly-NIPA/DMA gels in the separation of cytochrome from ovalbumin.

III. CONCLUSIONS

Weakly ionizable, thermosensitive gels can separate proteins based on differences in size and charge. For example, using only one separation stage, the concentration of cytochrome c relative to ovalbumin can be up to two-and-one-half times the relative concentration in the feed solution. In general, to achieve both high yield and high enrichment, the operating ranges of partition coefficient and mass fraction of gel are small because the maxima of the surfaces defined by these variables do not overlap. The relative concentration enrichment and solute yield in Process A depend on only the amount of gel and the solute partition coefficient. Process B depends weakly on the partition coefficient of the solute in the deswollen gel and on the difference in volume of the swollen and deswollen gel. The complex experimental dependence of the partition coefficient and swelling on material and solution properties can effect unexpected recommendations regarding the suitablility of a particular comonomer gel for a specific

separation process. For design purposes, it would therefore be desirable to estimate the swelling equilibria and the solute partition coefficient independently. Models for calculating swelling equilibria are available (4); the prediction of partition coefficients is discussed in Reference 7. Thermosensitive gels may provide potential process alternatives to ultrafiltration; but for any gel-based separation process, the major difficulty is synthesis of a gel which has the proper swelling and solute-interaction properties.

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Table 1

Process Calculations

GEL	PROCESS A (Cussler, et al) relative concentration (raffinate/feed) for 90% yield	PROCESS A (Cussler, et al) initial mass fraction gel	PROCESS B (Prausnitz, Blanch) maximum recovery	PROCESS B (Prausnitz, Blanch) relative concentration (raffinate/feed)
(I) NIPA; 15%T, 1%C	1.383	0.0316	100%	1.04
(II) NIPA/10%SA; 8%V, 1%C	1.09	0.00946	94%	0.97
(III) NIPA/10%DMA; 8%V, 1%C	1.08	0.00739	76%	1.33
(IV) NIPA/5%SA/5%DMA; 8%V, 1%C	1.04	0.0114	71%	1.12

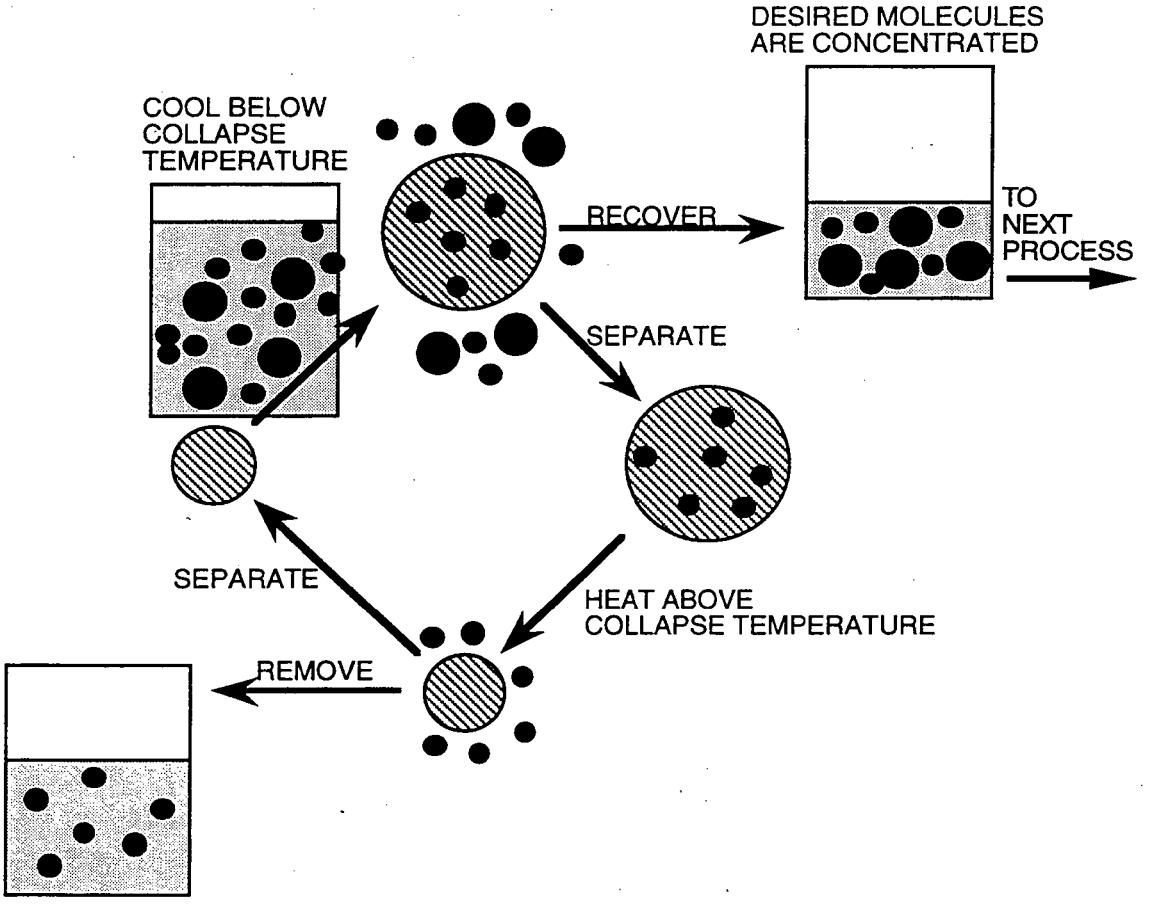

Figure 1a. Process A proposed by Cussler et al to use thermosensitive gels to concentrate solutes. In this process, because the solute of interest is too large to penetrate the gel, it is concentrated in the raffinate solution.

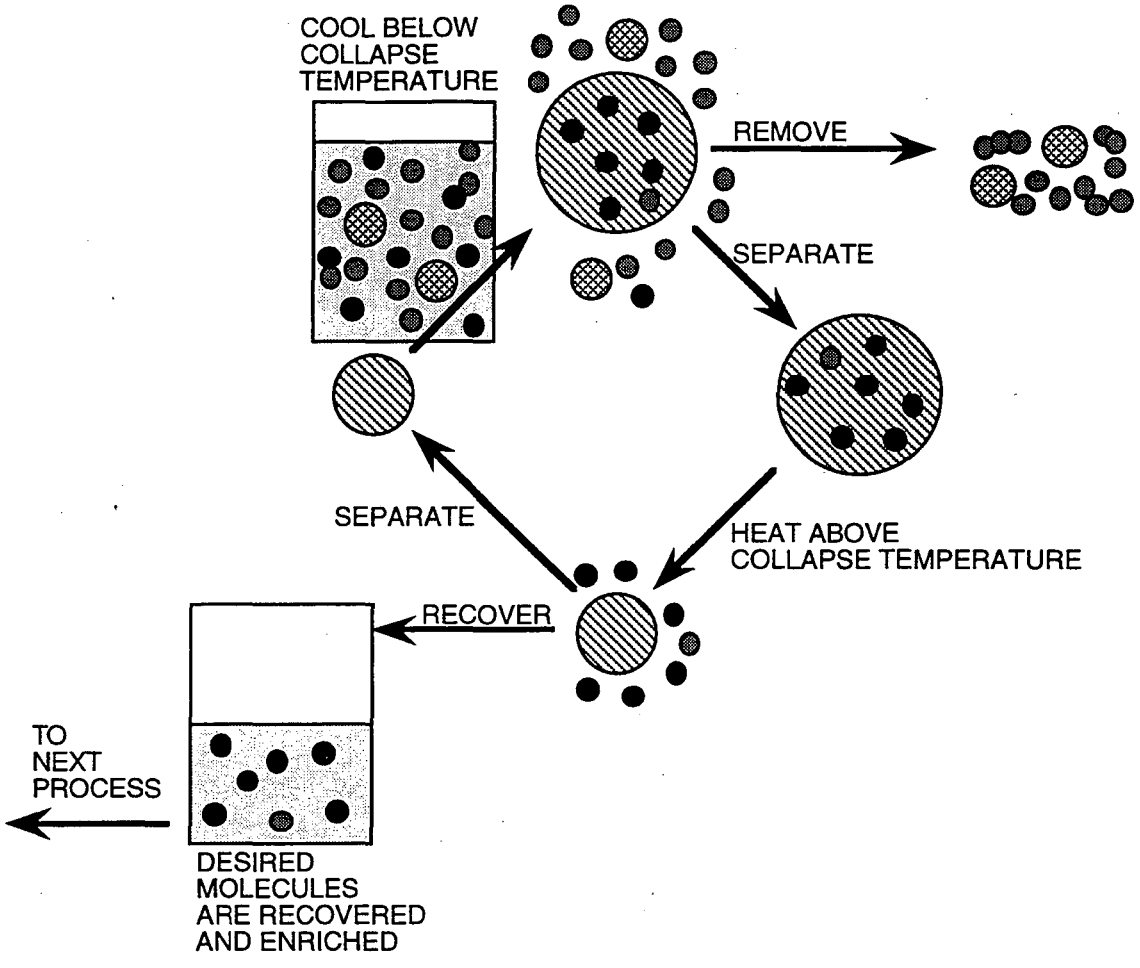

Figure 1b. Process B proposed by Prausnitz, Blanch et al to use a temperature- and/or pH-sensitive hydrogel to extract a solute of interest. In this process, the solute of interest partitions preferentially into the swelling gel. The gel is removed from the feed solution and collapsed under conditions which favor release of the solute of interest. The solute is recovered in the extract solution.

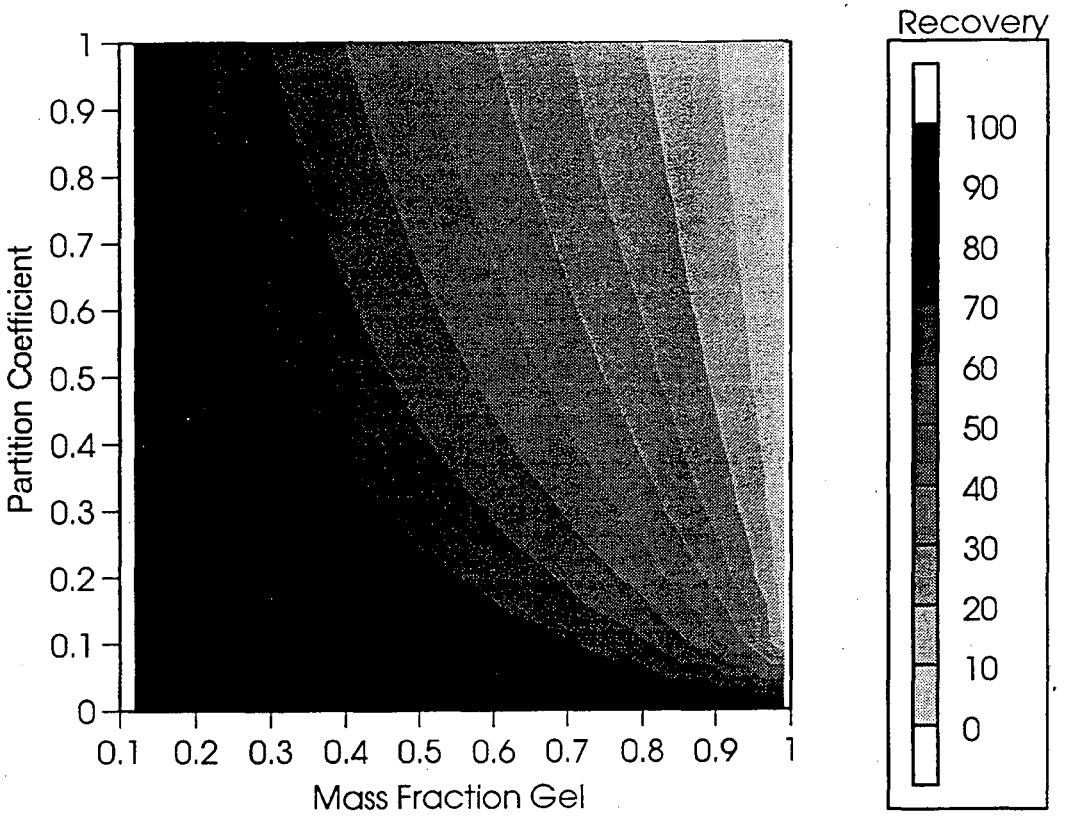

Figure 2. Effect of partition coefficient and mass fraction of swollen gel (before separation of the gel and the raffinate solution) on the recovery of a solute in Process A. The solute of interest is concentrated in the raffinate solution. The region of high recovery lies toward the corner of low partition coefficient and low gel mass fraction.

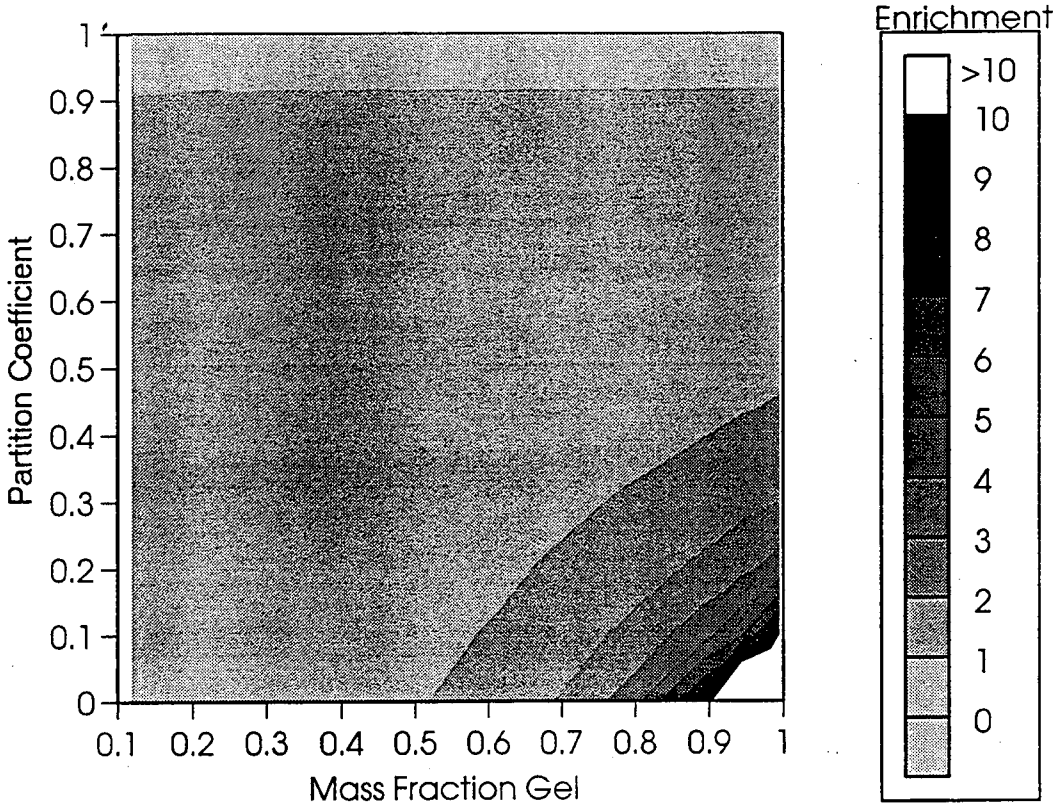

Figure 3. Effect of partition coefficient and gel mass fraction on concentration enrichment of a solute relative to the feed solution in Process A. The region of high enrichment lies in the extreme corner of low partition coefficient and high mass fraction of swollen gel.

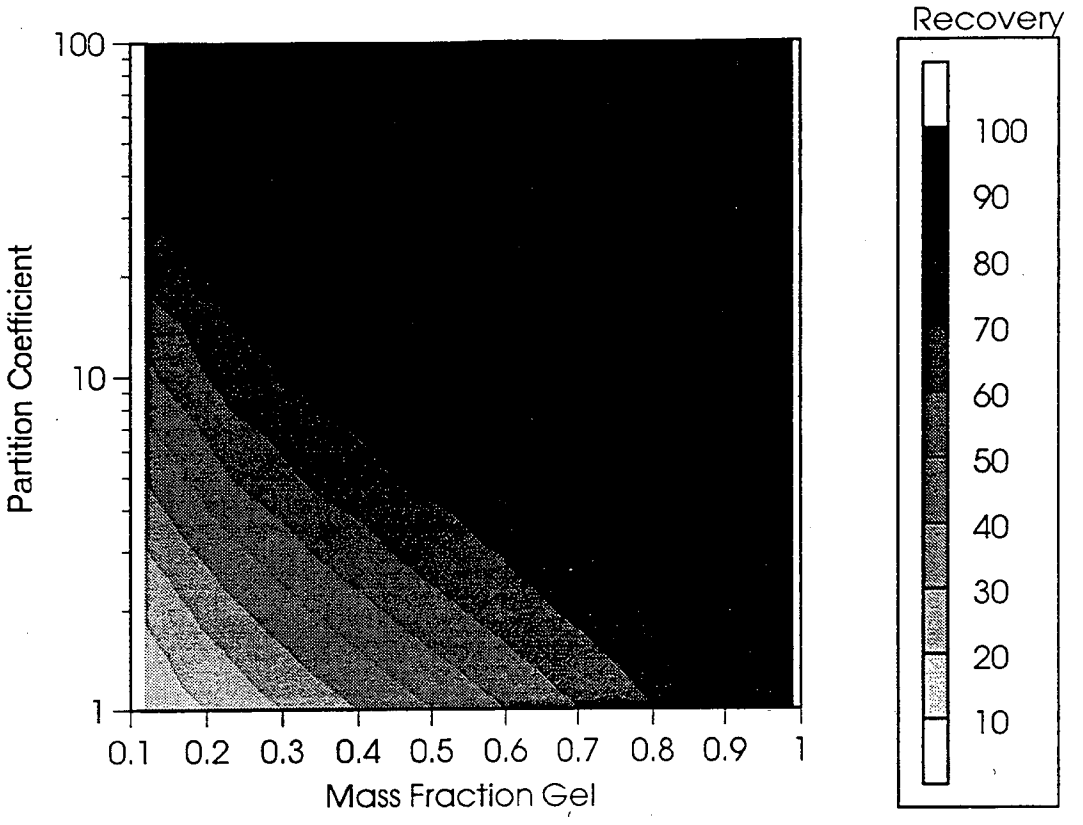

Figure 4. Effect of partition coefficient and mass fraction of swollen gel on the recovery of a solute in Process B. The solute of interest is recovered in the extract. The partition coefficient plotted is for the solute partitioning into the gel below the collapse temperature; the partition coefficient for the solute into the gel above the collapse temperature is constant at its optimal value of zero. The ratio of swelling of the collapsed gel to the swollen gel was set at 1/12. The region of high recovery lies now toward high partition coefficients and high mass fractions of swollen gel.

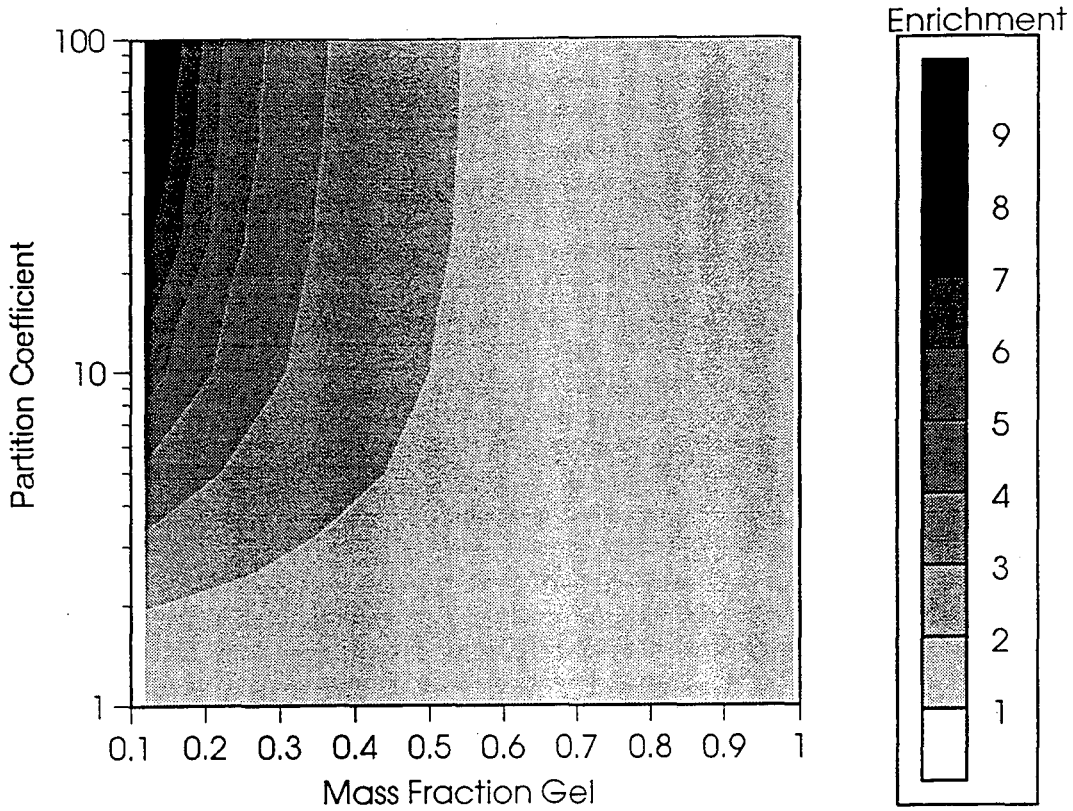

Figure 5. Effect of partition coefficient and mass fraction of swollen gel on the enrichment of a solute in the extract relative to the feed solution in Process B. Enrichment increases as the partition coefficient increases and mass fraction of swollen gel decreases. The partition coefficient of the solute into the collapsed gel is zero, and the ratio of swelling of the collapsed gel to the swollen gel is 1/12, as in Figure 4.

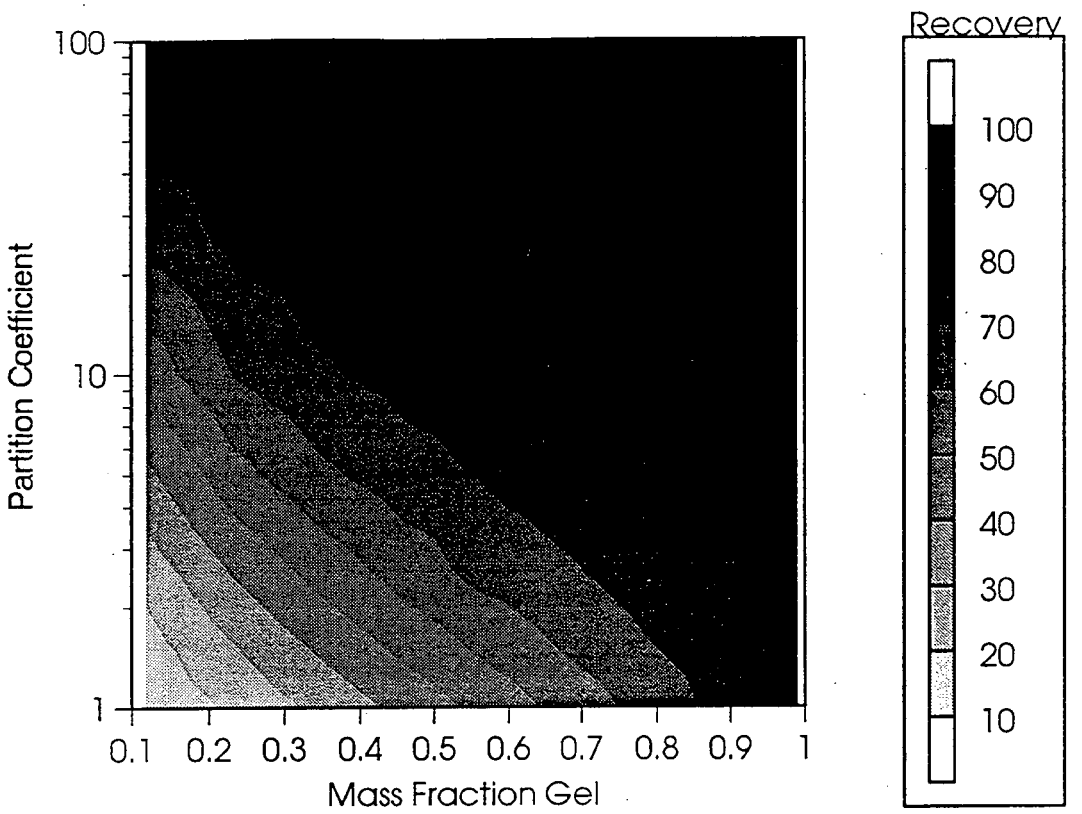

Figure 6. Effect of partition coefficient and mass fraction of swollen gel on the recovery of a solute in Process B. The figure is analogous to Figure 4, except that the partition coefficient of the solute in the collapsed gel is 0.5 instead of 0. The increase in partition coefficient of the solute in the collapsed gel reduces the area of high recovery, as shown by comparison to Figure 4.

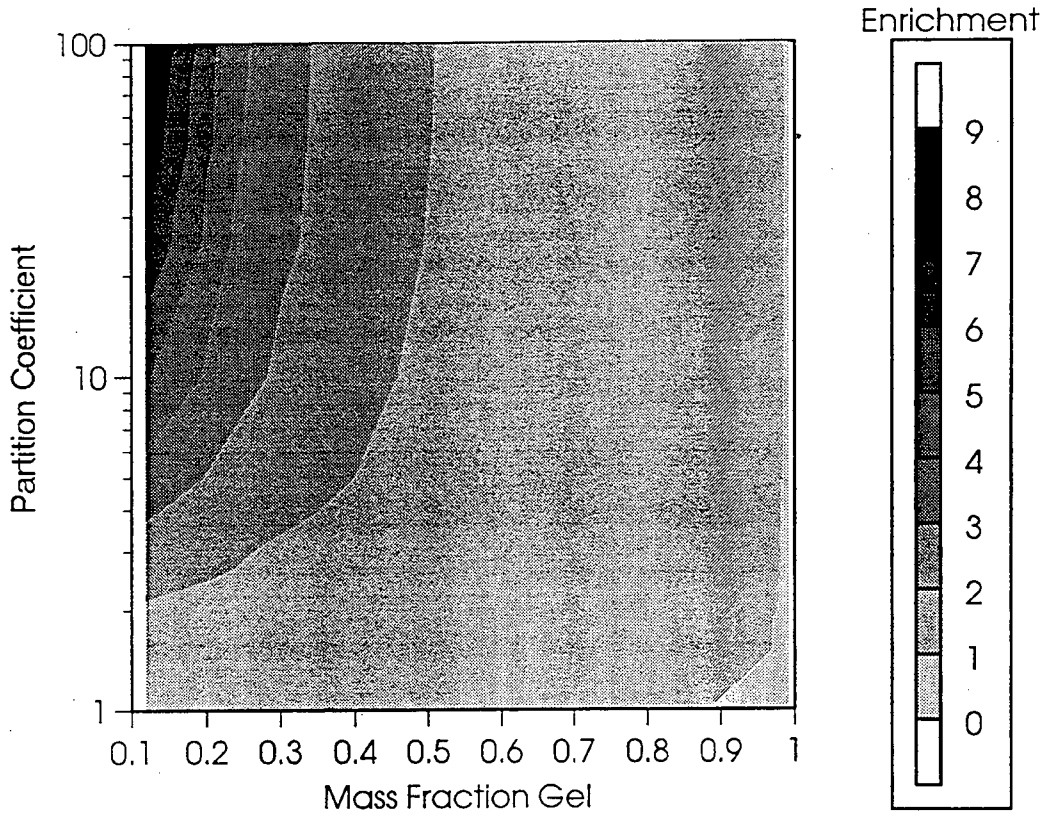

Figure 7. Effect of partition coefficient and mass fraction of swollen gel on the enrichment of a solute in the extract relative to the feed solution in Process B. The figure is analogous to Figure 5, except that the partition coefficient of the solute in the collapsed gel is 0.5 instead of 0. The increase in partition coefficient of the solute in the collapsed gel reduces the area of enrichment, as shown by comparison to Figure 5.

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