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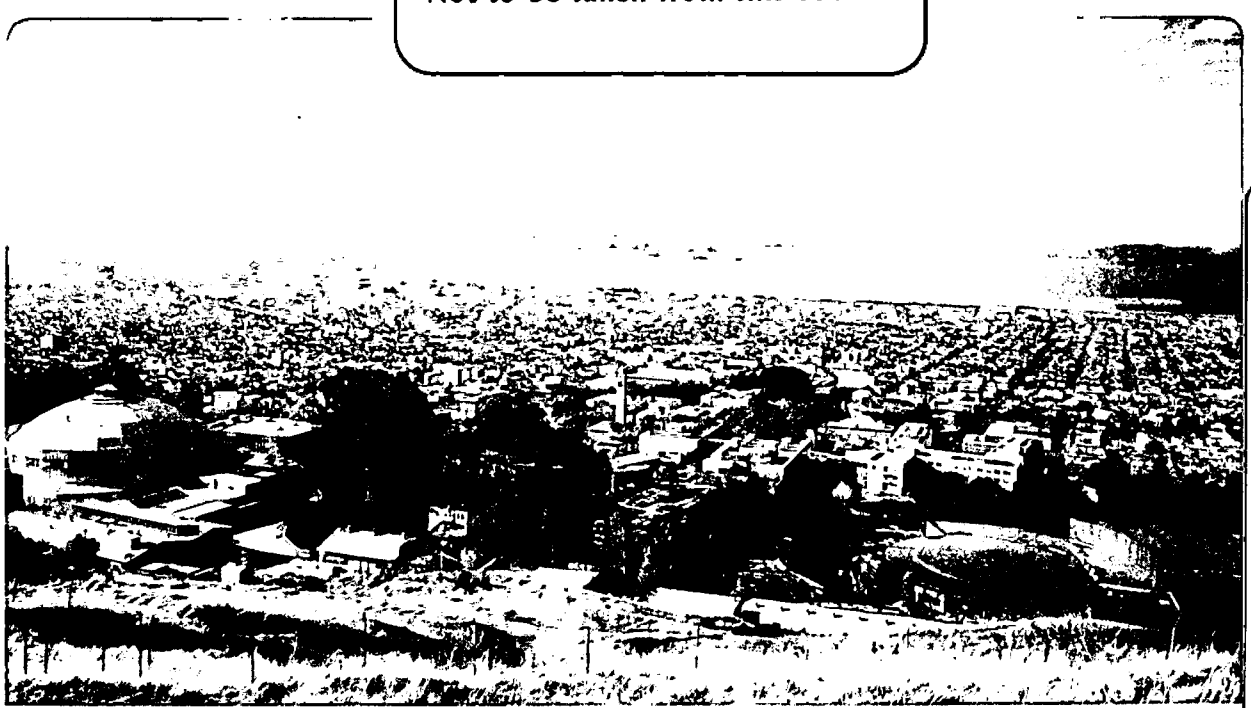

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CROSSLINKED GELS AS WATER ABSORBENTS IN SEPARATIONS

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ABSTRACT

A summary is presented of experimental and theoretical applications of hydrogels as water absorbents in separation operations. Proposed concentration schemes are described and briefly discussed. Fundamentals of gel chemistry, swelling, and selectivity are outlined. Published reports of experiments using swelling hydrogels for separations are reviewed. Theories to describe equilibrium gel swelling are presented and discussed.

I. INTRODUCTION

Concentration and recovery of solutes from dilute aqueous solutions are recurring problems in biotechnology. Examples include recovery of high-value pharmaceuticals from genetically-engineered cells and the concentration of biomolecules in fermentation broths. Large-scale production of common chemicals via fermentation is often not competitive with more traditional production methods because of the high cost of concentrating the desired product from dilute aqueous solutions (1).

Often concentration and/or recovery is desired without complete separation of individual solution components. Reverse osmosis and ultrafiltration provide two commonly used methods for concentration by solvent removal and for purification of macromolecules by removal of low-molecular-weight solutes. In these methods, an applied pressure forces the solvent (i.e., water) through a membrane. Thus, solutes unable to permeate the membrane are retained. However, a layer of solute-rich fluid or gel often builds up on the surface of the membrane, which inhibits solvent flux. Buildup of the layer, referred to as concentration polarization, can become the dominant resistance in ultrafiltration.

A convenient alternative to ultrafiltration may be provided by gels as water absorbents; gels avoid the expense and difficulties associated with high-pressure operation. The separation is based on the uptake of water and low-molecular-weight solutes by the gel, leaving behind a concentrated solution of larger molecules. Gel chemistry can be altered to provide a desired degree of selectivity.

On the large scale, competitiveness of a gel-based separation depends on the cost of regeneration and recycling of the gel, especially when the cost of gel replacement is high. For small-scale operations, recycling of the

gel may be unnecessary. Regenerable as well as non-regenerable gels have been studied for solute concentration by several researchers. Most attractive are the so-termed reversible hydrogels, which undergo order-of-magnitude volume transitions (collapses) in response to small changes in environmental variables such as temperature (2,3,10,22), ionic strength (2,3,10,20), pH (2,3,24), co-solvent ratio (2,3,11), electrical field strength (3), pressure (4), etc... These gels can be easily regenerated by slight changes in solution conditions (e.g. temperature), thus avoiding energy- and/or time-consuming processes such as repeated washings with various solvents and high-temperature vacuum drying.

Choice of a gel depends on the specific application. However, the following properties are of general importance:

- Mechanical Properties:
  The gel must be physically stable to withstand handling and regeneration procedures. It should also be easy to separate from the raffinate; for example, it should not be sticky.
- Chemical Stability:
  The gel should not decompose, dissolve or react with solution components.
- Reproducibility:
  Minimal hysteresis should occur in swelling behavior. Hysteresis is usually not a problem for mechanically stable gels.
- Absorbency:
  High equilibrium water content of the gel reduces the amount of polymer necessary and can cut process costs. The gel must swell significantly in the solution to be concentrated. Addition of a polyelectrolyte copolymer often significantly increases swelling capacity.
- Pore Size:
  Gels must have pore sizes appropriate to exclude or include desired solutes.
- Chemistry:
  If selectivity due to molecular interactions is desired, appropriate chemical groups or ligands must be incorporated in the gel.
- Kinetics:
  If separation time is crucial, size, geometry and chemistry of the gel particles must favor quick swelling.

Unfortunately, the above properties cannot be easily predicted; they must usually be determined experimentally. Gel swelling can be predicted

with varying degrees of accuracy by several theoretical models; however, as yet, no fundamental model exists to predict possible partitioning of a solute between solution and gel.

II. GEL-BASED CONCENTRATION SCHEMES

Three concentration processes have been proposed, as illustrated in Figure 1. Flodin, Vartak, Anderson, and others were the first to suggest the use of gels as a method for macromolecular separation (5-7). According to their procedure (Figure 1a), dried gel in the form of sticks is added to the solution to be concentrated. The gel is allowed to swell, imbibing water and low-molecular weight solutes. The gel is then removed from the solution and discarded or regenerated. Concentration is achieved by using the gel to remove water from the feed.

Cussler and co-workers proposed the use of reversible hydrogels as an alternative to ultrafiltration (Figure 1a) (8,9). Gel in its collapsed state is added to the feed solution and allowed to swell, absorbing water and small molecular-weight impurities. The swollen gel is then recovered from the now-concentrated raffinate and collapsed by shifting the pH (pH-sensitive gels) or raising the temperature (temperature-sensitive gels). As the gel collapses, it expels the imbibed water and solutes, regenerating the gel. The gel is again ready for contact with feed solution.

The third scheme, proposed by Blanch, Prausnitz and co-workers, takes advantage of solute partitioning (10). The process is carried out as in Cussler's method; however, the chemical nature of the gel is engineered such that desired molecules partition into the gel (Figure 1b). The gel is then collapsed under conditions that favor the release of the desired solutes from the gel. This scheme requires careful consideration of gel chemistry to select a gel which is selective for a solute(s) and, in addition, has the required swelling properties.

Experiments using gels for concentration and for separation have thus far been carried out only at the laboratory scale. However, it is likely that the process could be adapted from a batch mode to a semibatch or continuous mode, as in a continuous stirred tank reactor with introduction of the gel at the inlet and solid-liquid separation at the outlet.

While offering several important advantages over ultrafiltration, large-scale implementation of these processes faces several possible difficulties. Depending on the size of gel particles and their surface affinity for water, entrainment of solution during solid/liquid separation will reduce efficiency. Undesired adsorption of solutes onto the gel may require an extra step to regenerate the gel. New gel will need to be added to replenish that lost in the handling process. The maximum solids/liquid ratio permitted for operation as a semicontinuous process may necessitate

a multistage process to achieve the desired degree of concentration. These and other factors have yet to be studied before commercial-scale operations can be considered.

III. GEL CHARACTERIZATION

A hydrogel is a cross-linked polymer network swollen in water. Depending on chemical composition, gels may be prepared in aqueous or organic solutions or on a solvent-free basis. Co-monomers are added to alter chemical and mechanical properties. In some cases, gel properties are highly dependent on preparation conditions, e.g. initiator amount, temperature, solvent (11). However, once a preparation technique has been determined, gels can be made reproducibly. Gels are usually described using the following variables:

% T = molar percentage of total monomer at preparation
% C = molar percentage of crosslinker
% CM = molar percentage of co-monomer

Rigidity of the gel is strongly influenced by %C as well as %T. Increasing %C increases the number of bridges between the polymer chains, resulting in a more tightly bound network. Increasing %T increases the number of physical chain entanglements. To obtain a quantitative measure of the contribution of such entanglements, an effective crosslinking density can be measured by performing stress-strain measurements on the gels (12). Pore-size distributions can be measured by size-exclusion chromatography (13) or by direct visualization via Transmission (11) or Scanning (14) Electron Microscopy (TEM and SEM).

Gels made of strongly hydrophilic monomers (such as acrylic acid) are often too fragile and break easily. Incorporation of more than a few percent of a strongly ionized monomer, such as methacrylamidopropyl trimethyl ammonium chloride (MAPTAC), can also produce a weak gel. To obtain a more rigid gel, the %T and/or %C can be increased to form a tighter network. Alternatively, small amounts of a more hydrophobic monomer (e.g. a methacrylate) strengthen the gel. However, the capacity of the gel to absorb water decreases sharply when hydrophobic monomers are added or when entanglement becomes large.

Mechanical strength of acrylamide-based hydrogels can be increased not only by copolymerization but also by the formation of interpenetrating networks. For example, Mukae, et. al., have studied synthesis and characterization of an interpenetrating network of poly(ethylene oxide-dimethyl siloxane-ethylene oxide) and poly N-isopropylacrylamide (15). The presence of the oxide-siloxane-oxide network does not shift the

collapse temperature of N-isopropylacrylamide, but it does significantly affect maximum equilibrium swelling.

In the last decade, reversible phase transitions have been observed in hydrogels. Phase transitions are manifested in the form of a large volume collapse. These volume transitions are related to lower critical (consolute) solution temperature (LCST) phenomena in solutions of uncrosslinked polymers. A critical solution temperature is a minimum or maximum temperature at which solution behavior changes from complete to partial miscibility. Figure 2 shows the phase behavior for a general polymer solution; various forces determine the transition between two-phase and one-phase behavior. The upper critical solution temperature (UCST) in region A is due to differences in polymer-solvent miscibility and can be predicted by Flory-Huggins theory (16). The UCST in region B is due to entropic effects of mixing. The LCST in region C is due to compressibility differences between polymer and solvent. Finally, the LCST in region B is due to order-disorder transitions such as the stability of orientation-dependent hydrogen bonds. Gels consisting of polymers which exhibit an UCST undergo a phase transition from a collapsed state to a swollen state as temperature increases. Similarly, gels consisting of polymers which exhibit a LCST undergo the reverse transition; the gel collapses with rising temperature. At the transition temperature, it is possible for two gel phases to coexist, as observed by Hirotsu (17). The transition may be continuous (18,22,24,42) or discontinuous (9,19). Addition of a co-monomer changes the position and the magnitude of this transition.

IV. FACTORS AFFECTING GEL SWELLING IN AQUEOUS SOLUTIONS

The most important factor influencing the quantity of water absorbed by a gel is the chemical nature of the polymer. Hydrophilic polymers, such as acrylamides, may swell to the order of hundreds of times their dry weight (20), while hydrophobic polymers, such as methacrylates, swell only on the order of ten times their dry weight (21). Polyelectrolyte gels swell more than their non-ionic counterparts due to the extra osmotic force of water trying to enter the network. In reversible copolymer gels, gels with a greater ionic content (greater % charged co-monomer) display larger swelling transitions (22). In addition, the transition temperature of a thermo-reversible gel rises with incorporation of ionizable groups into the polymer forming the gel (22).

Decreasing %C or %T creates a looser network, allowing the gel to swell more. However, if %C or %T is significantly reduced in aqueous polymerization, no network will be formed during polymerization because the chains are too far apart. At intermediate levels of initiator, increasing the percent initiator increases the swelling capacity by producing a more

homogeneous gel. At high initiator levels, too many chains are formed at once, and no gel results. At a low initiator level and high %T, opaque clumps may form in the gel. These clumps, termed "popcorn polymerization", are high-density pockets formed when radicals in free-radical polymerization become "trapped". Clumps are rigid and diminish the swelling capacity of the gel. Popcorn polymerization may be reduced by any technique which enhances mobility of free radicals; for example, decreasing %T or introducing chain-transfer agents (11).

The presence of solutes may increase or decrease the swelling capacity relative to that in pure water (10,23). This effect may be related to the lowering of water activity. Swelling of polyelectrolyte gels is highly dependent on the ionic strength of the solution. As ionic strength increases, polyelectrolyte gels deswell. This effect is more pronounced with greater ionic content of the gel. The deswelling effect is attributed to screening of the charges on the gel network which repel each other and contribute to expansion of the gel. In gels with large volume transitions, increasing ionic strength consequently decreases the magnitude of the transition (22).

Swelling of gels incorporating a weak electrolyte is also sensitive to pH. Acidic gels swell more at high pH (basic conditions), and basic gels swell more at low pH (acid conditions). Increasing the ionic strength decreases the sensitivity of swelling to pH, again because of charge screening. At pH's where the gel is more ionized, the transition temperature for reversible gels is slightly higher, and the magnitude of the volume transition is also greater (24).

Reversible gels are extremely sensitive to the particular environmental variable which induces their collapse. Collapse transitions can be induced by temperature, pH, ionic strength, electrical field, UV radiation (25,26), solvent composition, or pressure. The collapse is often over orders of magnitude in degree of swelling.

Swelling kinetics are most influenced by geometry of the particles; the smaller the particle, the quicker it swells. However, for practical applications, the advantage of minimizing particle size is partially offset by entrainment between small particles when the gel is removed from solution. Hydrogels can easily be prepared in a variety of sizes. Particles in the submicron size are made by precipitation polymerization (27).

Several mechanisms for gel-swelling kinetics have been proposed. For example, Gehrke and Cussler postulated that swelling is determined by diffusion of water molecules into the network (28). Alternatively, Tanaka reported that collective diffusion of the network in water and bulk counter-flow of water through the network determine gel swelling (61).

In visual studies of temperature-dependent poly-N-isopropyl acrylamide/sodium acrylate gel spheres, Tanaka observed that kinetics of

swelling depends on the final temperature (62). If the gel swells through the volume transition regime (final temperature less than the collapse temperature), two polymer relaxation rates (one fast, one slow) are present. Kinetics of gel collapse through the transition exhibits three stages; initial, rapid deswelling, a plateau region where the gel stops swelling, and a resumption of deswelling at a slower rate.

Swelling in ionizable gels is determined also by the rate at which the ionizable groups of the network are charged. Therefore, swelling can also be determined by boundary-layer diffusion and ion exchange. For example, for polyacrylamide/sodium methacrylate gels swelling in basic solution, Gehrke and Cussler found that the rate of swelling increased when the solution was stirred (28). At low ionic strengths, Donnan equilibria retards hydroxide ions (which ionize the gel) from entering, thereby slowing the swelling process. At high ionic strength, the Donnan effect is no longer important (28).

V. GEL SELECTIVITY

The usefulness of a gel as a concentration device is linked to its ability to exclude certain solutes and imbibe others. Efficiency is related primarily to an interplay of steric and intermolecular forces.

Solutes too large to diffuse into the pores of a gel will be excluded. Thus, solute exclusion is a function of molecular weight, as in size-exclusion chromatography: large molecular-weight solutes are more efficiently excluded. Steric interactions are a function also of molecular shape; for example, compact solutes such as urea and sucrose are excluded less efficiently than polyethylene glycol of similar molecular weight (29). Hydrolyzed polyacrylamide will exclude neutral solutes greater than 3 nm in diameter with an efficiency of at least 80% (8).

Solute exclusion is enhanced by increasing %C, as the network becomes tighter. For example, the efficiency of exclusion of Vitamin B12 (MW 1355) is doubled when %C is increased from 1 to 6% in poly-N-isopropylacrylamide (12). The tradeoff, however, is that the swelling capacity declines with increasing crosslink density, necessitating the use of more polymer to take up the same amount of water. For low-molecular-weight solutes which do not interact significantly with the network, the effect of crosslink density on exclusion efficiency at a set %T can be correlated using the following equation (30), derived from excluded volume theories (66):

$$\frac{C_r/C_f - 1}{m_f/m_r - 1} = 1 - e^{-cv} \qquad (1)$$

where v is the volume of the solute, c is the crosslink density, C_f is the concentration of solute in the feed solution, C_r is the concentration in the raffinate solution, m_f is the mass of the feed solution, and m_r is the mass of the raffinate solution.

Increasing %T has a similar effect on size exclusion because chain entanglements increase, but the effect of %T cannot be correlated with the above equation. In reversible gels, swelling equilibria are sensitive to solution conditions (temperature, molality, etc...), and therefore pore sizes are similarly sensitive.

Solutes which interact with the gel can present an advantage or a disadvantage to gel-based separations. If a gel is used to concentrate macromolecules in the raffinate phase, efficiency will be severely compromised by any adsorption of macromolecules to the surface of the gel. For example, positively charged solutes, such as the protein lysozyme at pH 7, cannot be concentrated in the raffinate with hydrolyzed polyacrylamide because the lysozyme adsorbs onto the gel (11). Addition of a gel-washing step can recover the adsorbed molecules, but this results in a dilution of the adsorbed solute, which is undesirable. If, however, separation is to be achieved by absorption of and subsequent expulsion of a solute(s) (i.e., in the extract), favorable interactions with the gel are desired.

Little is known about the fundamentals of gel-solute interactions. Most experimental data come from gel chromatography; these data indicate that electrostatic and hydrophobic interactions are important (1,31,32).

Charged solutes small enough to enter the gel pores are expected to partition via Donnan-type exclusion. While it is observed that species oppositely charged with respect to the gel matrix partition into the gel, data for polyvalent solutes (such as dyes) cannot be correlated using ideal Donnan equilibrium, even at concentrations on the order of 10^{-4} M (10,29). With increasing ionic strength, electrostatic interactions become less important as charges become increasingly screened.

At higher salt concentrations, hydrophobic interactions become relatively more important (32). These interactions form the basis for hydrophobic-interaction chromatography (HIC) of proteins. Unfortunately, there is no *à priori* means to calculate this effect.

Solute concentration also affects partitioning between a solution and a gel phase. In some cases, higher solute concentration leads to higher partition coefficients; in other cases, the opposite is observed (10,33). The volume fraction of gel with respect to surrounding solution also seems to have an effect on the partition coefficient (10), although this has not yet been investigated.

VI. NON-REVERSIBLE GELS FOR CONCENTRATION OF DILUTE SOLUTIONS OF MACROMOLECULES

The use of a gel as a means of concentration was first suggested by Flodin, et. al., in 1960 (5). Flodin used 50-100 mesh Sephadex G-25 gel (a crosslinked dextran) to concentrate cellulases fifteen-fold from culture media of *Polyporus versicolor*. One gram of dry gel was added per 10 milliliters of the culture medium, stirred for ten minutes and separated by centrifugation. The gel was then washed, and the wash water added to the centrifugate. The centrifugate was submitted to two additional concentration steps. Approximately 90% of the cellulases were recovered by this method. The dextran gels were regenerated by washing in water and in ethanol, with subsequent drying at 80 °C. No differences could be detected in the performance of the regenerated gel as compared to the fresh gel.

Urinary proteins were concentrated in a three-stage process using P-6 Bio Gel by Anderson, et. al. in 1978 (7). In each stage, dry, 50-100 mesh gel was added to the urine sample. The resultant slurry was stirred briefly and loaded into a Büchner funnel lined with filter paper. The funnel was then set into a beaker and centrifuged at 10 °C for 5 minutes at 2000 rpm. Two-dimensional gel electrophoresis (ISO-DALT) of the resultant urine concentrate was carried out with results that compared favorably to samples concentrated by dialysis and lyophilization. Gel was regenerated by repeated washings, first in distilled water, then in methanol. After washing with methanol, the gel was dried at 36 °C in a vacuum oven.

Various proteins and macromolecules were concentrated using gel sticks of polyacrylate by Vartak, et. al. , in 1983 (6). Enough dry gel sticks (300 mg each) were added to 50 ml of solution to absorb 90% of the water within 4-5 hours at 25 °C or below. The sticks used could absorb 170 ml of water per gram of dried gel in pure water. Solutes included subtilisin inhibitor, cytochrome c, myoglobin, soybean trypsin inhibitor, bovine serum albumin (BSA), ferritin, CM cellulose, starch and DNA. Loss of solute varied from 7 to 20%; nearly a ten-fold concentration was achieved. From a cellulase/glucose solution, Vartak, et. al., were also able to concentrate cellulase thirty-fold with only 10% loss while simultaneously absorbing 75% of the glucose into the gel, thereby achieving a separation of enzyme and hydrolysate product. Reuse of the gel sticks was not reported.

VII. REVERSIBLE GELS FOR CONCENTRATION OF DILUTE SOLUTIONS OF MACROMOLECULES

Reversible gels have the added advantage that they can be easily regenerated (cycled) by small changes in environmental variables. In this way, the same gel is used repeatedly with minimum investment of

materials and energy. Investigations into the use of reversible hydrogels for separations have been made primarily by Cussler and co-workers (Andrews, Freitas, Gehrke, Stokar, Trank, Varberg) (8,9,28,29,30,34,39) and Prausnitz, Blanch and co-workers (Baker, Beltrán, Hooper, Sassi) (10,20,22,24). Both pH and temperature-sensitive acrylamide-based gels have been studied. Swelling equilibria for some of these gels are outlined below, followed by possible specific applications to separations.

VIII. GEL SYSTEMS

To our best knowledge, the gels examined for use as concentration devices have all been of the acrylamide family, co-polymerized with various monomers to impart hydrophobic or ionic character. Polymerization of these acrylamide gels is carried out by free-radical polymerization in water. Methylene bis-acrylamide is used as a crosslinking agent, and sodium metabisulfite, ammonium persulfate and tetramethylethylenediamine (TEMED) have been used as initiators. For details of gel polymerization, see references 9, 11, and 20.

A. HYDROLYZED POLYACRYLAMIDE

Polyacrylamide is well-known in applications in chromatography and gel electrophoresis. Upon hydrolysis of the amide groups to carboxylic acid groups, a polyacrylamide gel can undergo volume transitions with respect to pH, temperature, solvent concentration and electric field strength (34). Hydrolysis can be catalyzed by either acid or base; however, larger swelling capacities are achieved when catalyzed by base because of increased hydrolysis. The magnitude of the swelling transition is greater for higher degrees of hydrolysis.

In 1984 (8), Cussler, Stokar and Varberg proposed a separation scheme using hydrolyzed polyacrylamide. Bio-Gel P-6 was hydrolyzed in a solution of 0.5 M $NaHCO_3$ for one day at 50 °C. The hydrolyzed gel could then be collapsed upon the addition of acid. Alternatively, polyacrylamide can be prepared using techniques for gel electrophoresis and subsequently hydrolyzed. Gel volume versus pH is shown in Figure 3 (34).

B. POLY N-ISOPROPYL ACRYLAMIDE AND COPOLYMERS

Poly- N-isopropyl acrylamide (NIPA) gels exhibit LCST behavior near 34 °C. The effect of temperature on the volume transition has been reported both as continuous (22) or discontinuous (9), depending on the method of preparation. Poly-N-isopropyl acrylamide gels for separations have been examined by Freitas, et. al. (9) and Sassi, et. al. (10).

The inclusion of an ionizable co-monomer can greatly increase the maximum swelling capacity. Beltrán, et. al., have studied the swelling of copolymers with the strong electrolyte methacrylamidopropyltrimethyl

ammonium chloride (MAPTAC) and with weak electrolytes such as sodium acrylate (SA) and dimethylaminoethyl methacrylate (DMA)(28).

Figure 4 shows swelling capacity of NIPA/MAPTAC gels in water as a function of temperature. As the percent MAPTAC incorporated increases, collapse of the copolymer gel becomes more continuous and occurs at higher temperatures. The magnitude of the gel collapse also increases with electrolyte content. The effect of 0.1M NaCl on gel swelling is shown in Figure 5. At this salt concentration, differences in swelling capacity due to charge are greatly diminished.

Swelling capacity is shown in Figure 6 for NIPA/SA and NIPA/DMA gels as a function of pH (24). While both copolymers contain 10% comonomer, the maximum swelling capacities are different, probably because of chemical differences in the two monomers. The effect of pH on the temperature-induced collapse of NIPA/SA is shown in Figure 7. As the gel becomes more ionized (higher pH), the collapse transition occurs at higher temperatures and becomes more gradual, as observed for NIPA/MAPTAC copolymers.

C. POLY N,N-DIETHYLACRYLAMIDE/SODIUM METHACRYLATE

Linear poly- N,N-diethylacrylamide (DEAA) also exhibits LCST behavior near 29-30 °C (63). Freitas et. al. prepared a copolymer of 97 or 99% N,N diethylacrylamide and 2% sodium methacrylate for investigation of solute exclusion. Addition of the sodium methacrylate increases the collapse temperature to 55 °C. Swelling of the DEAA/sodium methacrylate gel is shown as a function of temperature in Figure 8 (35).

D. POLYACRYLAMIDE GELS CONTAINING AFFINITY LIGANDS

For maximum solute selectivity, affinity ligands may be incorporated into the gel matrix, just as in affinity chromatography. A dual-functional affinity gel was synthesized by Ruann, et. al., by co-polymerizing acrylamide, acrylic acid, and acryloyl 6-aminohexyl Cibacron Blue F3G-A (an affinity ligand) (36). The resultant gel has two binding sites for an enzyme (carboxylic acid groups and Cibacron Blue) providing more binding sites than the affinity gel Blue Sepharose CL-6B.

Grafted gels of polyacrylamide/methacrylamide and poly-amino acids may also show promise as affinity gels, although no experimental results have been reported for these polymers (37). For example, N-acetyl-D-alanyl-D-alanine is a binding ligand for the antibiotic vancomycin, used for treating infections of bacteria resistant to β-lactam antibiotics. Incorporation of D-alanyl-D-alanine into an acrylamide gel could result in a affinity gel for vancomycin recovery (38).

IX. SOLUTE CONCENTRATION

Most of the experimental data on solute concentration are for single species in aqueous solution at low ionic strengths (< 0.01M). This type of data simplifies initial development of a solute partitioning theory, as competing interactions from other solutes are not present, and it demonstrates the potential usefulness of gels. However, from a practical standpoint, knowledge of partitioning at high ionic strengths with multiple solutes present provides a more realistic test of the feasibility of using gels to concentrate solutions for practical applications.

A. CONCENTRATION OF SINGLE SPECIES

Concentration of several solutes using acrylamide-based gels has been studied by Cussler and co-workers and Blanch, Prausnitz and co-workers. Their results are summarized in Table 1 (8,9,10).

Cussler and co-workers (Andrews, Freitas, Gehrke, Stokar) have studied concentration of proteins in the raffinate phase using temperature-sensitive and pH- sensitive gels. As the concentration of molecules relies on solute exclusion, Cussler, et. al., report data in terms of the percent efficiency of exclusion, η, defined by

$$\eta = \frac{C_r/C_f - 1}{m_f/m_r - 1} \times 100 \qquad (2)$$

where C_f is the concentration of solute in the feed solution, C_r is the concentration in the raffinate solution, m_f is the mass of the feed solution, and m_r is the mass of the raffinate solution.

Blanch, Prausnitz and co-workers (Sassi, Baker) are studying partitioning of proteins into temperature-sensitive and pH-sensitive gels. In this case, separation is based on selective partitioning of solutes into the gel. Data are reported in terms of the partition coefficient, K, defined by:

$$K = \frac{m^{gel}_{solute}}{m_{gel}} \times \frac{m_{solution}}{m^{solution}_{solute}} \qquad (3)$$

where m_{gel} is the mass of the swollen gel, $m_{solution}$ is the mass of the solution surrounding the gel at equilibrium, m^{gel}_{solute} is the mass of solute in the gel phase and $m^{solution}_{solute}$ is the mass of solute in the solution phase.

Table 1 reports both partition coefficients (K) and efficiencies (η). The partition coefficients reported do not attempt to make any distinction between bulk partitioning and surface adsorption. Because the objective of

Cussler and co-workers is totally to exclude the solute, the value of the efficiency is negative if the solute partitions favorably into the gel. Additional exclusion data for polyethylene glycols of various molecular weights in NIPA and DEAA/NaMA gels can be found in reference 9.

Although the data are limited, several important characteristics can be observed. Solutes oppositely charged with respect to the gel matrix partition favorably into the gel; those with the same charge as that of the matrix are excluded. For example, the NIPA/3% MAPTAC gel is positively ionized. Bovine serum albumin at pH 8 is negatively charged and interacts favorably with the gel (K=18.5), but at pH 3, BSA is positively charged and is virtually excluded (K=0.2). The addition of a co-monomer to increase swelling capacity probably allows larger solutes to enter; BSA is totally excluded from NIPA at pH 3 but is able to enter slightly into NIPA/3% MAPTAC at pH 3. The addition of 0.1 M Na_2SO_4 appears to screen electrostatic interactions between BSA and NIPA/MAPTAC or NIPA/DMA gels; the partition coefficient of BSA is lower in the presence of the added salt.

In experiments with pH-sensitive hydrolyzed acrylamide, Stokar placed 0.7 to 0.1 grams of dry gel into a basket inside a centrifuge tube with 20 grams of basic solution; each solute was at a fixed pH (34). The grams of gel and solution added were weighed separately. As indicated in Stokar's PhD thesis, the tube was shaken on a wrist-action shaker until the gel swelled and then centrifuged at 1000 rpm for 5 minutes to separate the phases. Centrifugation at a higher rpm was found to squeeze a significant amount of water from the gel. Both gel and raffinate phase were weighed, and the volume of the gel calculated using density data.

In his evaluation of the effect of cross-link density on solute exclusion, Andrews followed a procedure similar to that of Stokar, except that the gel was placed in a 100-ml graduated cylinder with a screen piston (30). The raffinate was removed by pushing the piston against the gel, inverting the cylinder, and draining the liquid through the screen. Freitas, using temperature-sensitive gels, altered the procedure by removing the swollen gel from the graduated cylinder with a stainless-steel screen attached to a rod (35).

In experiments by Sassi, et. al, a weighed amount of dried gel (usually about 0.1 - 0.2 g) is added to 30 grams of solution containing solute, triply de-ionized water, 0.01 M citrate-phosphate buffer and 0.1 g/L sodium azide as an antibacterial agent. The covered beaker containing gel and solution is placed in a constant-temperature bath (35°C) or refrigerator (10 °C) until swelling equilibrium is reached, usually on the order of two days for swollen disks on the order of 1.5-2 cm in diameter and cm thick. It has been demonstrated that partitioning equilibrium is reached at least as quickly as swelling equilibrium (28). The gel disks are

then removed with a spatula, blotted dry and weighed. Concentration of the solute in the remaining solution is determined by UV absorbance.

B. CONCENTRATION OF MULTIPLE PROTEINS

Poly N-isopropylacrylamide gels have also been used to concentrate soy proteins from aqueous extracts of soybean flakes (39). The use of gels was proposed to replace the acid precipitation step in current soy-protein recovery processes. Dried gel held in mesh baskets was lowered into extracts made by soaking one part defatted soy flakes in five parts water for 30 minutes at pH 8-8.5. Enough dried gel was added so that half the solution volume was absorbed at 5 °C. Gel was collapsed at 40 °C.

For dilute solutions (5 %wt protein), soy proteins were almost completely rejected by the gel, resulting in a doubling of their concentration. Small molecular weight solutes, such as salts, sugars and phytins, partitioned almost evenly into the gels. However, in solutions of 15 weight percent protein, the protein was not completely excluded, reducing the efficiency of the process to half that for 5% protein solutions. In addition, the swelling capacity of the gel (at 5 °C) was 50 percent of that in the dilute solution, presumably due to the lower activity of water in the concentrated protein solution. Protein losses could be reduced by more than half by centrifugation of the gel as opposed to gravity drainage as a means of solid/liquid separation. Washing the gel with pure water or water/octanol also increased the amount of protein recovered.

C. CONCENTRATION OF ENZYMES USING AFFINITY LIGANDS

A dual-functional affinity gel, acrylamide/Cibacron Blue/acrylic acid, has been used to adsorb the enzymes pyruvate kinase (PK) and 3-phosphoglycerate kinase (PGK) from a yeast extract by Ruann, et. al. (36). In the experiments, 0.5 ml pre-swollen gel was added to one milliliter of extract at pH 6.5 (PK) or 7.2 (PGK) and allowed to stand for 45 minutes. The gel was then removed, centrifuged, and placed in fresh buffer at a higher pH to elute the enzyme for 30 minutes. The gel was eluted twice to obtain recoveries of PK on the order of 70%. Simply raising the pH did not elute PGK; 0.4M KCl had to be added to obtain a 64% recovery after two elution steps. While a significant amount of enzyme is not recovered, purification factors (a measure of how enriched the eluted protein is in active enzyme) are on the order of 2.25 for PK and 13.0 for PGK.

Use of an affinity gel can compete well with other laboratory enzyme-purification methods. Ruann reported that the use of affinity-ligand gels cut the normal purification time from several days to several hours, recovered 35% more enzyme, and produced a product with higher enzymatic activity.

D. VIRUS CONCENTRATION

Poly N-isopropylacrylamide gel has been used to concentrate avian influenza virus (A/Duck/613/MN/79) from allantoic fluid for storage of smaller fluid volumes (40). Recovery of virions averaged 75% (high-95%, low-57%) in approximately one tenth the original sample volume. Approximately 8 grams of dried poly N-isopropylacrylamide was added to 90 ml of allantoic fluid at 4 °C and allowed to expand for 90 minutes. Concentrated allantoic fluid was separated from the gel in a Büchner funnel, and the gel was washed twice with a 5-ml aliquot of hemagglutination inhibition buffer.

X. GEL SWELLING MODELS

Theories which predict swelling equilibria of gel/solvent systems are fundamental extensions of those for non-crosslinked polymer solutions. For example, Flory's classical theory of network swelling incorporates an elastic term into his well-known theory of polymer solutions (16). Because the polymer is present only in one phase (the gel phase) the chemical potential of the solvent (1) is the same both inside (') and outside (") the gel,

$$\mu'_1 = \mu''_1 \tag{4}$$

This equation is usually rewritten for the solvent as follows:

$$\Pi = 0 = - \frac{\mu'_1 - \mu''_1}{V_1} \tag{5}$$

The quantity Π can be thought of as a swelling pressure, which is zero at equilibrium where forces trying to expand exactly balance forces trying to contract the gel.

The task, then, is to develop a suitable expression for the Gibbs energy (G) of the gel/solvent system. The chemical potential, μ, is a partial derivative of G:

$$\mu_i = \left(\frac{\partial G}{\partial n_i} \right)_{T,P,n_{j \neq i}} \tag{6}$$

To calculate Π, we use the Flory-Rehner assumption of additivity:

$$\Pi = \Pi_{mix} + \Pi_{elastic} + \Pi_{ion} \tag{7}$$

Π_{mix} reflects contributions from polymer/solvent mixing, $\Pi_{elastic}$ reflects elastic contributions from network deformation, and Π_{ion} accounts for electrostatic contributions in polyelectrolyte systems. Models differ in their choice of expressions for Π_{mix}, $\Pi_{elastic}$, and Π_{ion}, and hence may be limited by these choices to describing only special situations. Flory's original model of network swelling cannot predict the volume collapse of temperature-sensitive gels because in Π_{mix} it does not take non-random interactions into account (41). In the following sections, a review is presented of the lattice-based models that have been used to predict gel swelling.

A. LATTICE MODEL (Flory)

Flory developed his classical model for network swelling for both ionized and non-ionized gels (16). The mixing term is taken from his lattice-based polymer-solution theory by setting the number of polymer molecules, n_2, to zero, as individual polymer molecules are absent from the network structure. The mixing term is a completely random term and cannot account for preferential interactions. The elastic term is taken from his theory of rubber elasticity. This term accounts only for linear deformations in three dimensions; i.e., each chain segment deforms with the same extension/compression ratio. This means of deformation is known as the affine network. The ionic term is derived assuming ideal Donnan equilibria for a dilute solution. Thus, the expression for the swelling pressure is (subscript 1 denotes solvent, subscript 2 denotes polymer):

$$\Pi_{mix} + \Pi_{el} + \Pi_{ion} = -\frac{RT}{V_1}\left[\ln(1-\phi_2) + \phi_2 + \chi\phi_2^2\right] - RT\left(\frac{\nu_e}{V_o}\right)\left(\phi_2^{1/3} - 0.5\phi_2\right) + RT\left[\frac{ic_2}{z} - \nu(c^*_s - c_s)\right] \quad (8)$$

where V_1 is the molar volume of the solvent, ϕ_2 is the volume fraction of the polymer, χ is a parameter characterizing the solvent/polymer interaction, ν_e is the effective number of chains in a network, i is the number of charges per chain, c_2 is the molar concentration of chains, z is the valence of the ion, ν is the number of chains in the network, and c^*_s and c_s are the molar salt concentrations outside and inside the gel.

Flory's theory provides the basis for many gel-swelling models. Because each site has only one interaction potential, the Flory model in its basic form can predict swelling equilibria for non-polar gels in non-polar solvents. As hydrogels may incorporate polar or charged side groups, and because the solvent (water) is polar, Flory's basic theory is not appropriate

for hydrogel swelling. In particular, it cannot predict LCST volume transitions observed in reversible gels.

B. LATTICE MODEL WITH REWRITTEN IONIC TERM (Hirotsu, Tanaka)

One modification of Flory's model is presented by Hirotsu (42); the ionic term is rewritten as (43)

$$\Pi_{ion} = vfkT\left(\frac{\varphi}{\varphi_o}\right) \tag{9}$$

where φ is the volume fraction of polymer in the swollen state, φ_o is the volume fraction polymer in the dry state, v is the number of chains per unit volume of the dry state and f is the number of counterions per chain.

Instead of fitting the Flory parameter, χ, directly from swelling data, Hirotsu replaces χ in the mixing term with an expression based on enthalpy and entropy differences between the polymer-polymer and polymer-solvent interaction ($\Delta H_{pp/ps}$, $\Delta S_{pp/ps}$):

$$\chi = \frac{\Delta H_{pp/ps} - T\Delta S_{pp/ps}}{2kT} \tag{10}$$

The parameters $\Delta H_{pp/ps}$ and $\Delta S_{pp/ps}$ were fit to Ito and Mizoguchi's data for aqueous, uncrosslinked NIPA (64), φ_o was estimated from the asymptotic value of the swelling ratio at high temperatures, and v was estimated from the amount of crosslinker. When these independently obtained values were used, Hirotsu, et. al., reported poor agreement between the model and experimental swelling equilibria. However, when $\Delta H_{pp/ps}$, $\Delta S_{pp/ps}$ and v are fit to swelling data, the model predicts swelling equilibria for NIPA-SA copolymers almost quantitatively (42).

C. LATTICE MODEL WITH NON-GAUSSIAN DISTRIBUTION AND POLYMER CHARGES (Ilavsky)

Various correction terms have been added to Flory's theory. For example, Ilavsky, et. al., proposed the addition of two terms (44). The Flory model assumes that the distances between points of crosslinking (junction points) follow a Gaussian distribution. Ilavsky proposes dividing the elastic contribution into Gaussian and non-Gaussian components. The non-Gaussian component provides an attempt to consider the finite

extensibility of the chains and is derived by assuming a Langevin distribution function. The non-Gaussian correction is:

$$\Pi_{el}^{NG} = -\nu_d RT\left(\frac{3}{5}\langle\alpha_o^2\rangle^2 \varphi_2^{-1/3} n^{-1} + \frac{99}{175}\langle\alpha_o^2\rangle^3 \varphi_2^{-1} n^{-2} + \frac{513}{875}\langle\alpha_o^2\rangle^4 \varphi_2^{-5/3} n^{-3} + \ldots\right) \tag{11}$$

where ν_d is the molar concentration of chains with respect to the dried volume of the gel, $\langle\alpha_o^2\rangle$ is the dilatation factor of the dried state, n is the number of statistical segments in the chain, and φ_2 is the volume fraction polymer in the swollen state.

Ilavsky's theory also attempts to take into account electrostatic interactions between charged groups fixed on the network. As the degree of swelling changes, so does the strength of repulsive interactions between these fixed charges. Katchalsky, et.al., derived an expression for the free energy of network charge interactions in a free polyelectrolyte solution assuming that the Debye radius of the ionic atmosphere is small compared to chain dimensions (45). Ilavsky adapted this expression for an ideal network with no defects or free ends. The contribution to Π from the electrostatic energy between charged groups on the chain is:

$$\Pi_{net.} = \frac{\nu_d N_a Z^2 i^2 e^2 \varphi_2^{4/3}}{3D\left(\bar{h}_o^2\langle\alpha_o^2\rangle\right)^{1/2}}\left[\frac{2.5\bar{A}}{1+\bar{A}} - \ln(1+\bar{A})\right]$$

$$\bar{A} = \frac{6h}{x\bar{h}_o^2} \tag{12}$$

where ν_d is the molar concentration of chains with respect to the dried volume of the gel, Z is the degree of polymerization of the chain, i is the ratio of the number of charges to the number of monomeric units, e is the unit charge, φ_2 is the volume fraction of polymer, D is the dielectric constant, $\langle\alpha_o^2\rangle$ is the dilatation factor of the dry state, h is the chain end-to-end distance of the deformed state, h_o^2 is the end-to-end distance of the reference state, and x is the inverse of the Debye radius of the ion atmosphere.

Ilavsky's model has been used to correlate swelling equilibria for various acrylamide-based hydrogels. It is able to reproduce observed

phase transitions if the interaction parameter, χ, is allowed to be a function of both temperature and concentration. The predictive power of the model is limited because the interaction parameter, χ, must be known as a function of composition and temperature.

D. LATTICE MODEL WITH HOLES (Marchetti, et. al.)

To compensate for the inability of the Flory model to predict lower critical solution temperatures as a function of pressure, Marchetti and Cussler have derived a model which can account for pressure effects (46,47). By introducing holes as a third component in the lattice, Marchetti, et. al., have made the lattice compressible. The introduction of holes requires two additional χ terms, one for solvent-hole interactions and one for gel-hole interactions. These parameters are not zero, but are functions of cohesive energy densities of the species. The Gibbs energy of mixing term then becomes (subscript s denotes solvent, g denotes gel and o denotes holes):

$$\frac{\Delta G_m}{kT} = n_s \ln\phi_s + n_g \ln\phi_g + v^*_s n_s \chi'_{sg}\phi_g + n_0 \ln\phi_0 + v^*_0\left[n_0\chi'_{s0}\phi_s + n_0\chi'_{g0}\phi_g\right]$$

$$\chi'_{sg} = \frac{1}{kT}\left[\left(P^*_s\right)^{1/2} - \left(P^*_g\right)^{1/2}\right)^2 + 2Z_{sg}\left(P^*_s P^*_g\right)^{1/2}\right]$$

$$\chi'_{s0} = \frac{P^*_s}{kT}, \quad \chi'_{g0} = \frac{P^*_g}{kT} \tag{13}$$

where n_j is the number of sites of type j, ϕ_j is the fraction of sites of type j, P^*_s and P^*_g are the cohesive energy densities of the solvent and gel, v^*_j is the volume of site j, and Z_{sg} corrects for deviations from the Hildebrand combining rule.

The elastic term remains the same as that in the Flory-Rehner model (affine network), in which the network chains transform linearly upon deformation (65), and no ionic term is incorporated. The theory has one adustable parameter, Z_{sg}. The parameters, v^*_s, v^*_0 and P^*_s are fit from vapor-liquid equilibrium data for the pure solvent (48). Cohesive energy density P^*_g can be obtained by differential scanning microcalorimetry on un-crosslinked polymer solutions, assuming that cross-links do not alter these energy densities, and $v(\phi_g{}^o)^{2/3}V_g{}^o$ is found from the elastic modulus of the gel. The equation of state for the gel system becomes

$$P = -kT\left\{\frac{1}{v_o^*}\left[\ln(v_o^*) + \left(1 - \frac{v_o^*}{v_s}\right)\phi_s + \phi_g\right] + \frac{\nu\phi_g^o}{v_g^*}\left(\frac{\phi_g}{\phi_g^o}\right)^{1/3} + \left[\chi'_{so}\phi_s + \chi'_{go}\phi_g\right](\phi_s + \phi_g) - \chi'_{sg}\phi_s\phi_g\right\} \quad (14)$$

where ν is the number of crosslinks, and superscript ^{o} denotes conditions at gel synthesis.

Marchetti and Cussler's model is the only one capable of predicting pressure effects on phase transitions, although the prediction is only qualitative. It is able to predict a first-order phase transition and correlate swelling equilibria for poly(N-isopropylacrylamide) and for poly(diethylacrylamide) at low pressures.

E. QUASI-CHEMICAL LATTICE MODEL (Hooper, et. al.)

The oriented quasi-chemical lattice model for gel swelling developed by Hooper, et. al., gives semi-quantitative predictions for the swelling of non-electrolyte and electrolyte gels in aqueous salt solutions (41). The model is based on a lattice framework and takes into account orientation-dependent interactions which give rise to LCST behavior in non-crosslinked, aqueous polymer solutions. Polymer segments and solvent molecules each occupy one site; thus, there is no account for pressure or individual ions in solution. Each segment is given one, two or three interaction sites: dispersion-force interaction sites, hydrogen-bond donating sites, and hydrogen-bond accepting sites. Interactions between segments and solvent molecules depend on the orientation of the sites because the surface potentials of each segment (site) are not equivalent.

The mixing term is that derived by Prange, et. al., using Guggenheim's quasichemical approximation (49) to describe aqueous, hydrophilic polymer solutions. The mixing term (Π_{mix}) then becomes (subscript 1 denotes solvent, 2 denotes polymer):

$$\Pi_{mix} = -\frac{RT}{V}\left[\ln\phi_1 + \phi_2 - \frac{1}{2}z_1^{\alpha}q_1\ln\left(\frac{\Gamma^{\alpha\alpha}_{11,pure}}{\Gamma^{\alpha\alpha}_{11,mix}}\right) - \frac{1}{2}z_1^{\beta}q_1\ln\left(\frac{\Gamma^{\beta\beta}_{11,pure}}{\Gamma^{\beta\beta}_{11,mix}}\right) - \frac{1}{2}z_1^{D}q_1\ln\left(\frac{\Gamma^{DD}_{11,pure}}{\Gamma^{DD}_{11,mix}}\right)\right] \quad (15)$$

where V is the molar volume, ϕ is the volume fraction, z^{α}_i is the number of sites per segment of type a, q_i is a surface parameter, and $\Gamma^{\alpha\beta}_{ij}$ is the non-random factor for an α–β contact between segments j and k.

The elastic term is derived from the constrained-junction theory which interpolates between the two commonly-used idealized cases: the

affine and phantom networks. In the affine network, the junction points (points of crosslinking) are not allowed to move about in space and each chain transforms linearly upon deformation. In the phantom network, junction points are allowed to move about freely regardless of the state of deformation of the gel. The constrained junction theory interpolates between these cases by restricting the extent to which junction points are allowed to fluctuate; this restriction accounts for the constraints of neighboring chains on movement of the points of crosslinking. Unlike the phantom and affine network theories, the constrained-junction theory should be able to account for the effect of %T as a constraint on gel swelling. The elastic term is

$$\Pi_{el} = -\frac{1}{V_1}(\Delta\mu_{1,elas}^{phant}(1-F) + \Delta\mu_{1,elas}^{aff}F)$$

$$\Delta\mu_{1,elas}^{aff} = RT\left(\frac{\phi_2^o}{X_c}\right)\frac{1}{\lambda}\left(2 - \frac{1}{\lambda^2}\right)$$

$$\Delta\mu_{1,elas}^{phant} = \frac{RT}{2}\left(\frac{\phi_2^o}{X_c}\right)\frac{1}{\lambda}$$

$$F = K(\lambda,k)/(1 - \lambda^{-2})$$

$$k = 0.25P\Phi_2^{o\,0.5}X_c$$

(16)

where X_c is the average number of segments per network chain, λ is the dilation ratio, P is a dimensionless parameter which characterizes the polymer and solvent, and $K(\lambda,k)$ is an arbitrary grouping of terms which take into account the degree of interpenetration of the network chains (50). Interpolation function F depends on dilation ratio λ, and on the constraints on junction fluctuations k.

The ionic term (for polyelectrolyte gels) is derived from ideal Donnan theory, thus ignoring ion-ion, polymer-polymer, and ion-polymer electrostatic interactions. The rationale is that for low concentrations of charged co-monomer, these contributions provide only second-order corrections. The ionic term then becomes

$$\Pi_{ion} = RT\sum_j (c_j^{gel} - c_j^{ext})$$

(17)

and for a solution with a single, univalent salt,

$$\frac{c_s^{gel}}{c_s^{ext}} = \frac{c_s^{ext}}{c_s^{gel} + \left(\frac{i\phi_2}{V_u}\right)} \qquad (18)$$

where i is the fraction of charged monomers and V_u is the molar volume of one monomer unit. The summation is over all mobile ions j. The concentration of ions in the gel is determined by ideal Donnan equlibrium. Deviations from ideality are neglected.

The above model contains only three adjustable parameters; the exchange energy parameters ω^{DD}, $\omega^{\alpha\beta}$, and $\omega^{\delta\alpha}$ in the mixing term. Values for these parameters are found by fitting swelling equilibria data for uncharged gels in water. The model of Hooper, et. al., has been applied to swelling of the polyelectrolyte, copolymer hydrogels acrylamide/MAPTAC and NIPA/MAPTAC. In most cases, the theory provides at least semi-quantitative agreement with experiment.

Figure 9 compares measured and predicted isothermal swelling equilibria in aqueous salt solutions for 15% T, 0.2%C polyacrylamide/MAPTAC gels with varying % MAPTAC (20). While the model predicts well the effect of salt concentration on isothermal swelling equilibria and the effect of percentage crosslinking (not shown), it provides only qualitative predictions of the effect of % T, as illustrated in Figure 10.

Figure 11 compares measured and predicted swelling equilibria in water as a function of temperature for NIPA/MAPTAC hydrogels with varying % MAPTAC (20). The model predicts semi-quantitatively the observed increases in the magnitude of the swelling transition and the transition temperature with varying %MAPTAC. However, the model is not able to predict the continuous volume transitions observed experimentally. Figure 12 shows the effect of salt concentration on the volume transition; again, the model does not fit well in the transition regime.

XI. SOLUTE-PARTITIONING THEORY

No predictive, molecular-level theory has as yet been developed to predict partitioning of solutes in hydrogel systems. There have been, however, a number of investigations of steric partitioning of "ideal" molecules (i.e., hard spheres, random-flight chains) into pores of different

geometries. Methods used have included virial expansions (51-56), integral-equation theories (57), Monte Carlo simulation (58) and density functional theory (59). Effects of concentration, size and electrostatics predicted by these models agree qualitatively with experimental observations.

Glandt has shown that partitioning of bovine serum albumin (BSA) into controlled pore glass treated to prevent protein adsorption is predicted well by Monte Carlo simulation of the movement of spheres into sponge-like networks (58). An equivalent solute size for BSA was taken as an average over all possible orientations of an ellipsoid. In general, however, only qualitative results have been achieved, owing to the drastic simplifications inherent in these models.

De Ruvo has derived an expression using Flory-Huggins theory for partitioning of dilute solutes into a swollen gel (60). However, de Ruvo's expression neglects steric or electrostatic interactions and assumes that interaction parameters χ are independent of polymer concentration. Polymer-solute, solvent-solute and polymer-solvent χ parameters are required. De Ruvo did not compare his model with experimental data.

Because of its importance in chromatography and ultrafiltration, there is a need for a predictive model for solute partitioning. However, partitioning is difficult to model because of the interplay between steric and interaction effects.

XII. CONCLUSIONS

Use of hydrophilic gels for concentration of macromolecular solutes shows promise as an alternative to ultrafiltration. Gels which undergo large volume transitions show the most potential for such use, as the gels can be easily regenerated. Some success in concentrating solutes has already been demonstrated at the laboratory scale; however, only a few reports exist in the literature. Large-scale application is not likely in the near future because the fundamentals of gel swelling and solute interactions with gels are as yet only partially understood.

Truly predictive models for swelling equilibria of reversible hydrogels and solute partitioning are required for development of a gel-concentration process. While several models are able to correlate swelling equilibria when interaction parameters as a function of composition and temperature are available, a theory that is able to predict behavior out of the range of existing experimentally-measured conditions would be far more useful. The quasichemical model proposed by Hooper, et. al., is a step in this direction as it uses parameters fit to non-ionic gels in water to predict swelling of ionic copolymers in salt solutions. While semi-

quantitative predictions can be obtained with the quasichemical model, it is clear that significant improvements are necessary.

Solute partitioning is even less well understood but is crucial to potential applications of gels as outlined in this chapter. While Monte Carlo simulations are presently helping to clarify steric effects in idealized geometries, there is no engineering-oriented, à priori method to predict quantitatively the effect of molecular interactions between solute and gel. Much research will be required to bring the use of gels as concentration devices from the experimental laboratory stage to industrial use.

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TABLE 1

EXPERIMENTAL DETERMINATIONS OF PARTITION COEFFICIENTS (K) AND EXCLUSION EFFICIENCIES (η)

Solute	Gel	K	η	pH	Ref.
Urea	NIPA	0.943	2.0	N.A.	2
(60)	DEAA/NaMA	0.931	3.0	N.A.	2
Caffeine	NIPA	4.1	-411.	7	3
(194.2)	NIPA/1% MAPTAC	1.41	-46.4	7	3
	NIPA/2% MAPTAC	1.8	-65.	7	3
	NIPA/3% MAPTAC	3.56	-227.	7	3
Sucrose (342)	HYDROLYZED AAM	7.0	5.9	in water	1
Vitamin B12	NIPA/10% DMA	0.88	9.5	3	3
(1355.4)	NIPA/10% SA	0.75	21.1	3	3
	NIPA/10% DMA	1.07	-10.1	5	3
	NIPA/10% SA	1.01	-2.46	5	3
	NIPA	0.603	32.2	N.A.	2
	DEAA/NaMA	0.79	15.4	N.A.	2
Lysozyme	NIPA	1.31	-37.	8	3
(14,100	NIPA/3% MAPTAC	0.88	7.33	8	3
	NIPA/10% DMA	1.87	-90.	8	3
	NIPA/10% SA	210.	-605.	8	3
	HYDROLYZED AAM	14.2	N.A.	7	1
Ovalbumin	NIPA	0.022	96.6	N.A.	2
(45,000)	DEAA/NaMA	0.05	84.3	N.A.	2
Hemoglobin	NIPA	0.0	100.	8	3
(64,500)	NIPA/1% MAPTAC	0.0	100.	8	3
	NIPA/2% MAPTAC	0.0	100.	8	3
	NIPA/3% MAPTAC	0.33	63.5	8	3
	NIPA/10% SA	43.	-1054.	5	3
	NIPA/10% DMA	0.16	83.	5	3
	HYDROLYZED AAM	N.A.	91	10	1
Bovine	NIPA	0.0	100.	8	3
Serum	NIPA/3% MAPTAC	18.5	-848.	8	3
Albumin		0.19	79.	8*	3
(66,000)		71.	-2376.	8**	3
	HYDROLYZED AAM	0.04	93.	8	1
	NIPA/10% SA	0.0	100.	8	3
	NIPA/10% DMA	10.0	-679.	8	3
		0.48	48.	8*	3
		5.9	-501.	8**	3
	NIPA	0.0	100.	3	3
	NIPA/3% MAPTAC	0.2	82.	3	3
	NIPA/10% SA	1.9	-94.	3	3
	NIPA/10% DMA	0.6	38.	3	3

Gelatin	NIPA	0.03	98.	N.A.	2
(660,000)	DEAA/NaMA	0.02	97.	N.A.	2
Blue Dextran	NIPA	0.025	97.	N.A.	2
(2,000,000)	DEAA/NaMA	0.0054	99.	N.A.	2
Monoclonal Antibody (160,000)	NIPA	0.087	88.	N.A.	2

NIPA: N-isopropylacrylamide
AAM: Acrylamide
SA: Sodium Acrylate
DMA: Dimethylamino ethyl methacrylate
NaMA: Sodium Methacrylate
DEAA: Diethylacrylamide
MAPTAC: Methacrylamidopropyl trimethylammonium chloride

* with 0.1 M Na_2SO_4
** at 35 Celsius
N.A.: not available

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FIG. 1. Schemes proposed for the use of gel as a concentrating agent. Figure 1a illustrates the method proposed by Flodin, Vartak, Anderson, Freitas, Stokar and Cussler; Figure 1b illustrates the method proposed by Blanch and Prausnitz. (Ref. 5-10)

FIG. 2. Temperature-dependent phase behavior for a hypothetical aqueous polymer solution. Four consolute solution temperatures are possible.

FIG. 3. Swelling behavior of hydrolyzed polyacrylamide in water as a function of pH (ionic strength = 0.0007 M). The gel expands greatly in alkaline solution. (Ref. 34)

FIG. 4. Swelling behavior in water for NIPA/MAPTAC copolymer gels. The gels are temperature sensitive, but do not exhibit discontinous volume transitions. (Ref. 22)

FIG. 5. Swelling behavior in 0.1 M NaCl for NIPA/MAPTAC copolymer gels. The effect of charge on swelling capacity is significantly reduced from that observed in Figure 4. (Note expanded scale as compared to Fig. 4) (Ref. 22)

FIG. 6. Swelling equilibria at 7° C and 0.01 M ionic strength for NIPA copolymer gels in aqueous citrate/phosphate buffer solutions. NIPA/SA gels are more highly swollen in alkaline solutions; NIPA/DMA gels are more highly swollen in acidic solutions. (Ref. 24)

FIG. 7. Temperature-dependent swelling equilibria in 0.01 M aqueous citrate-phoshphate buffer solutions for NIPA gel at pH 6 and NIPA/SA gels at pH 3, 5, and 8. (Ref. 24)

FIG. 8. Swelling behavior in water for NIPA and DEAA/NaMA gels. The DEAA/NaMA gel has a more continuous collapse transition. (Ref. 35)

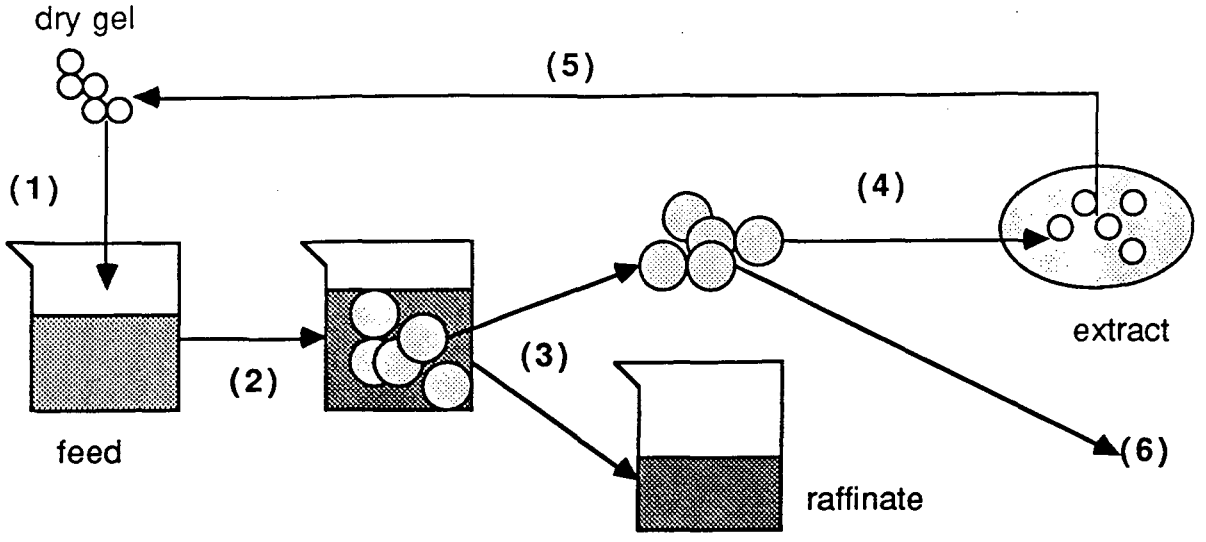
FIG. 9. Measured and predicted swelling equilibria for acrylamide/MAPTAC copolymer gels with varying concentrations of charged monomer using the model of Hooper, et. al. Predictions are based on known composition and structure parameters and on exchange energies determined from polyacrylamide gel swelling in water. (Ref. 20)

FIG. 10. Measured and predicted swelling equilibria for acrylamide/MAPTAC copolymer gels prepared at varying %T (monomer concentration) using the model of Hooper, et. al. Predictions are qualitative. (Ref. 20)

FIG. 11. Measured and predicted swelling equilibria for temperature-dependent NIPA/MAPTAC gel in water using the model of Hooper, et. al. The model predicts discontinous transitions for charged gels, but continous transitions are observed experimentally. (Ref. 22)

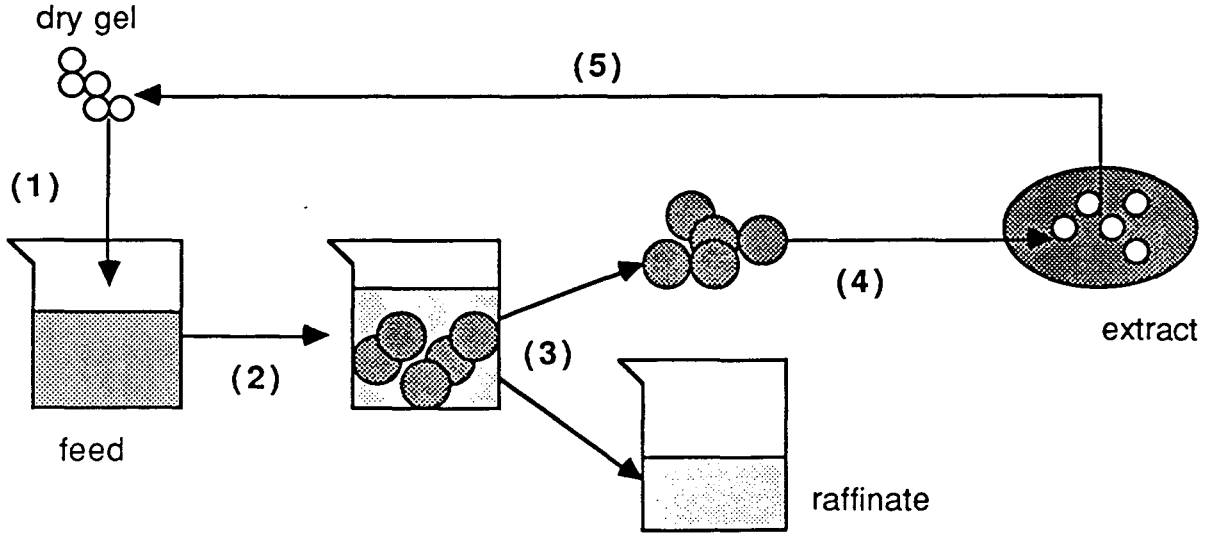
FIG. 12. Measured and predicted swelling equilibria for a temperature-dependent gel of composition 96% NIPA and 4% MAPTAC in water and aqueous NaCl solutions using the model of Hooper, et. al. The continuous transitions in salt solutions are not predicted by the model. (Ref. 22)

Figure 1a



(1) Dry gel is added to feed solution.
(2) Gel swells, absorbing water and low molecular weight solutes.
(3) Gel and solution phases are separated. Desired solutes remain in raffinate phase.
(4) Gel is collapsed by increasing temperature or shifting pH.
(5) Gel is returned to step (1) in its collapsed, wet state. OR
(6) Gel is discarded.

Figure 1b



(1) Dry gel is added to feed solution.
(2) Gel swells, absorbing water and desired solutes.
(3) Gel and solution phases are separated.
(4) Gel is collapsed by increasing temperature or shifting pH. Desired solute recovered in extract.
(5) Gel is returned to step (1) in its collapsed, wet state.

Figure 2

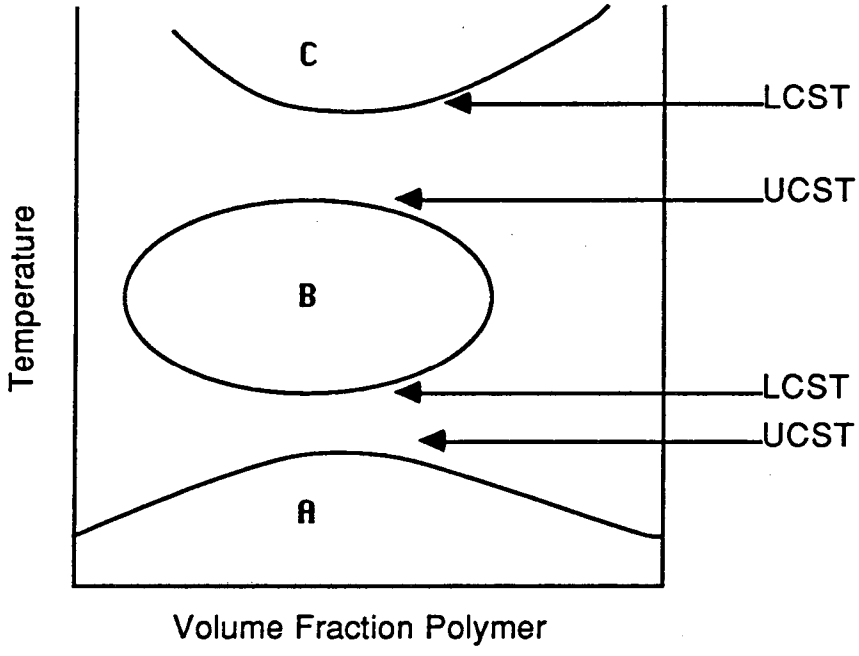

C
LCST
UCST
B
LCST
UCST
A
Temperature
Volume Fraction Polymer

Figure 3

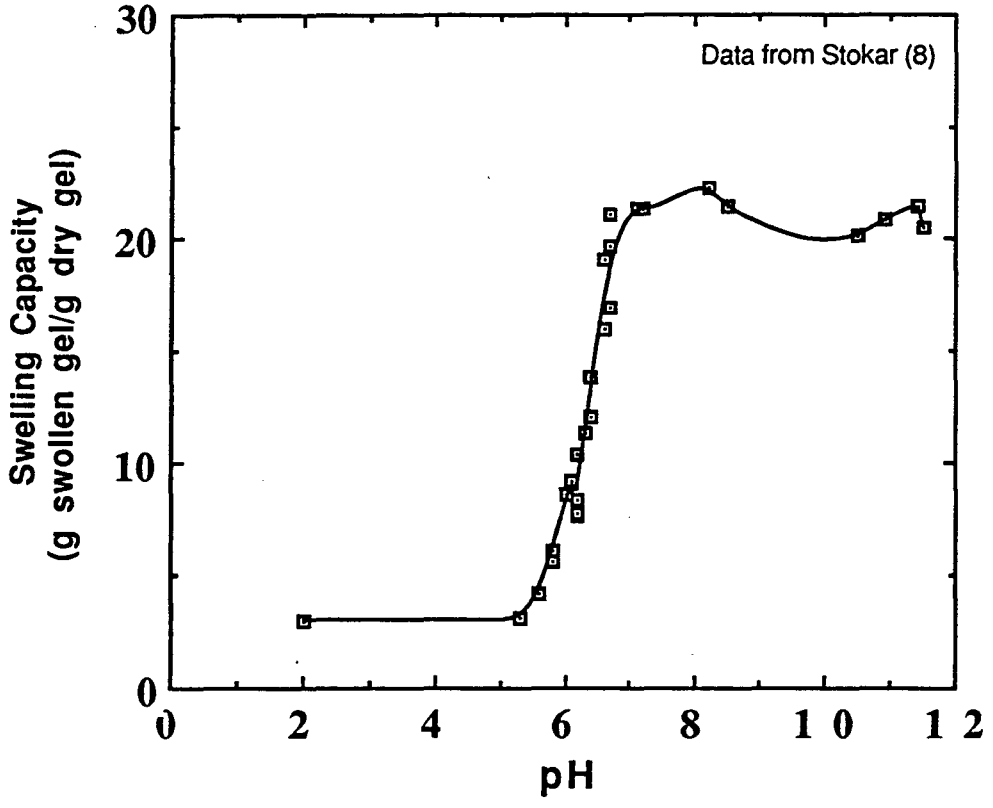


Figure 4

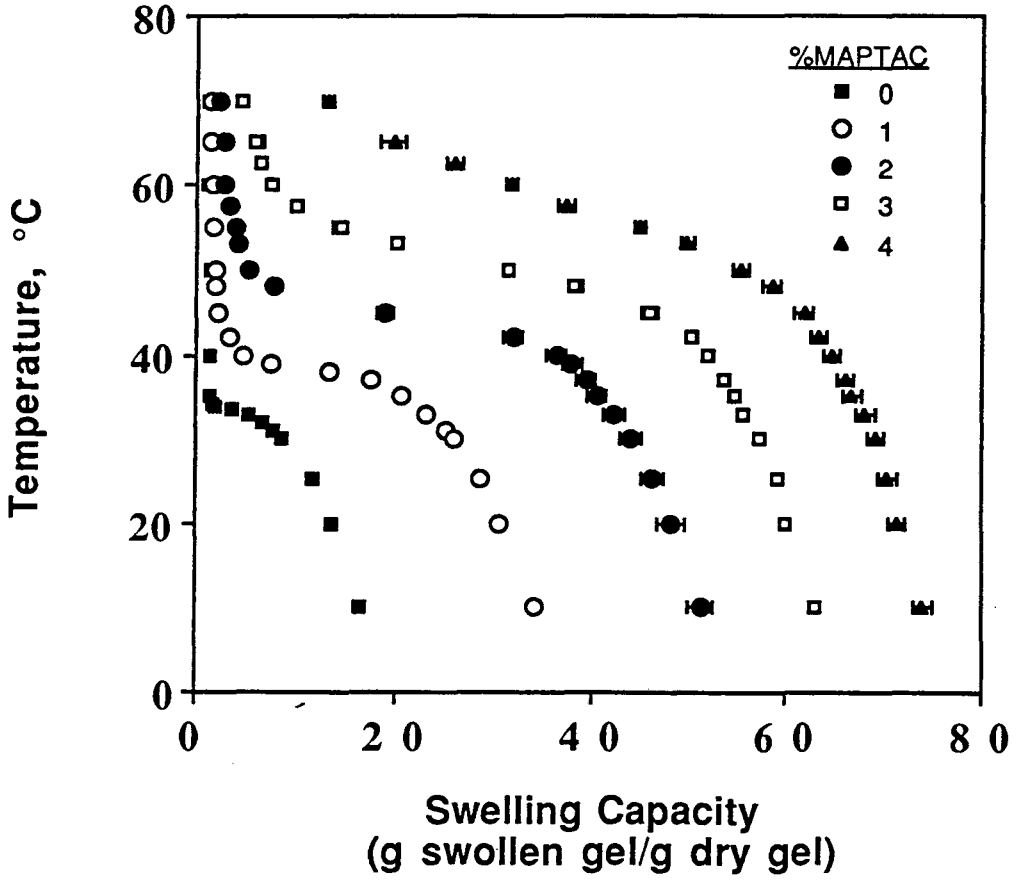


Figure 5

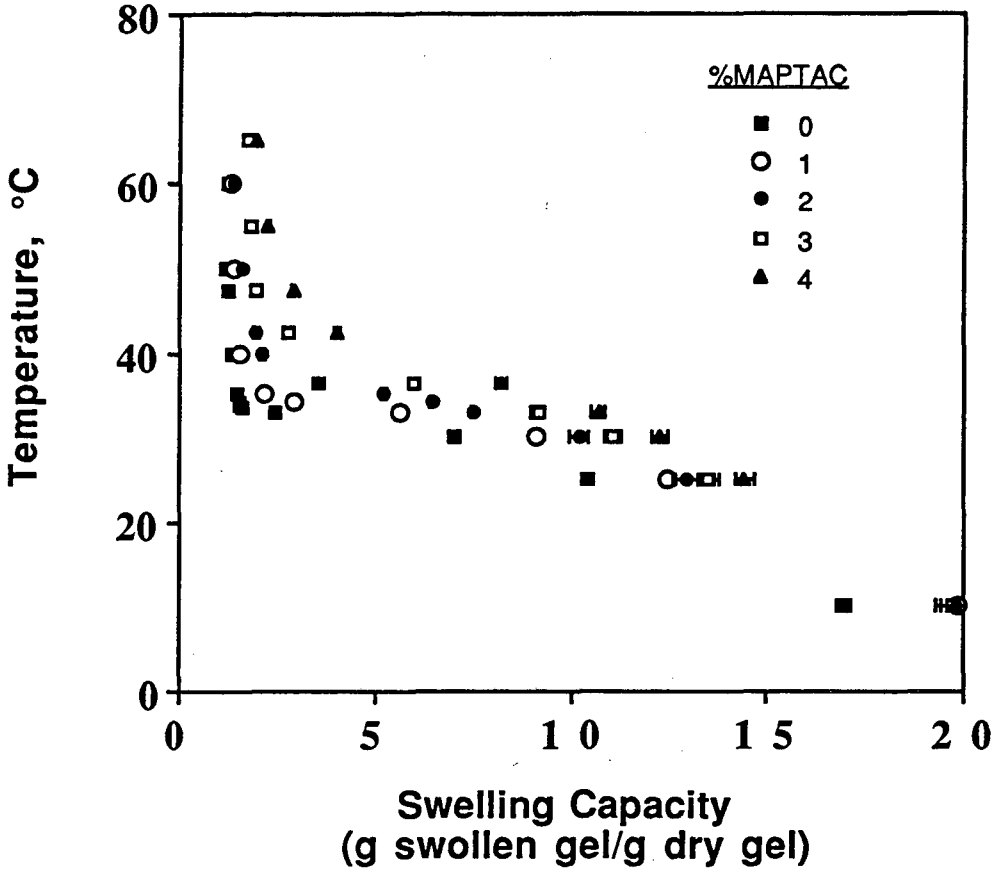


Figure 6

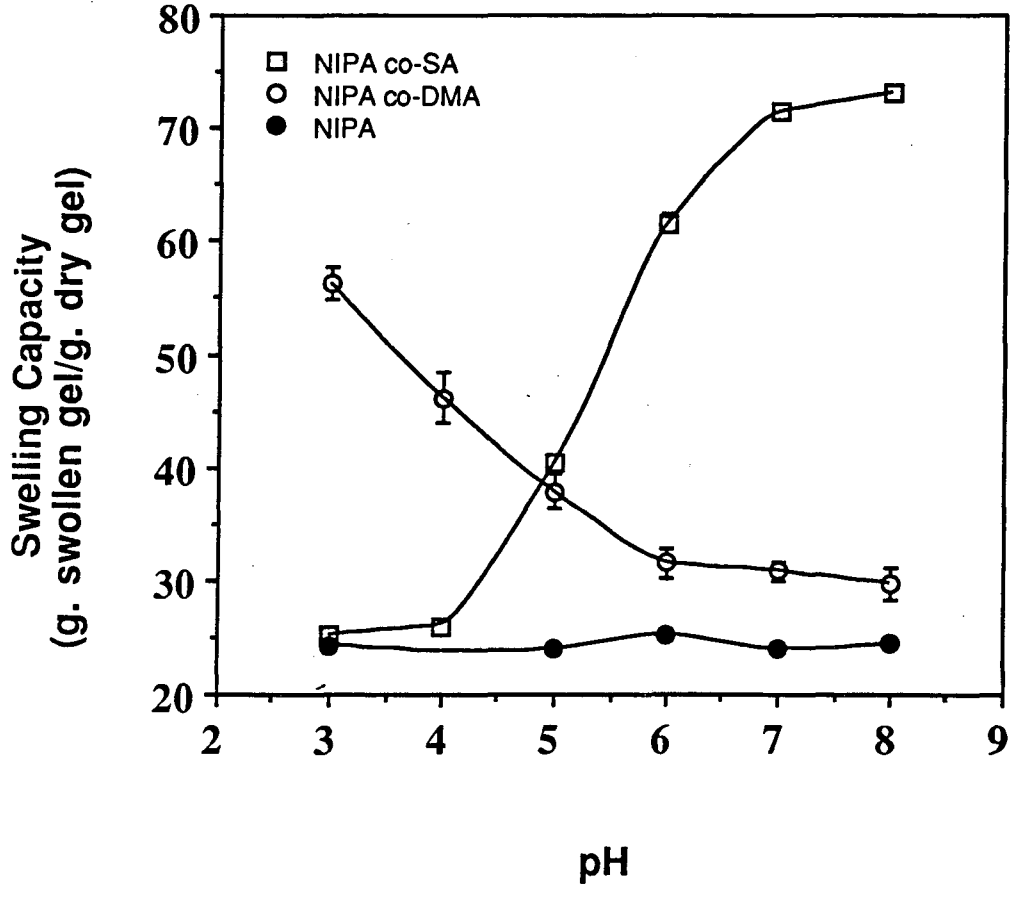


Figure 7

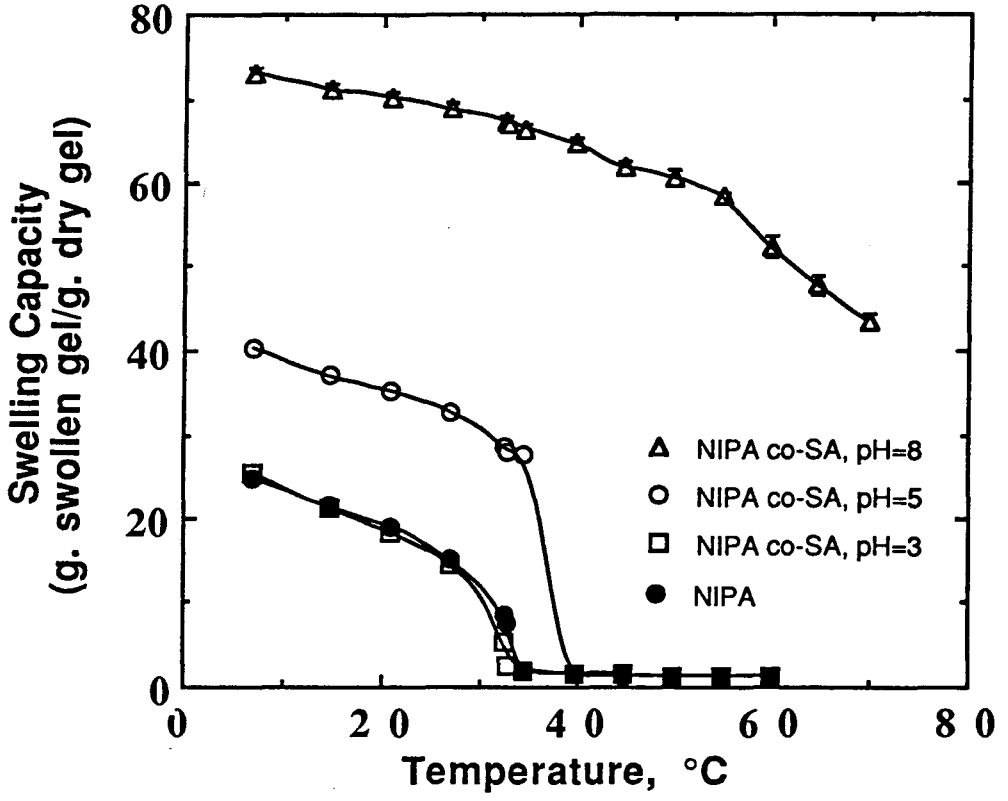


Figure 8

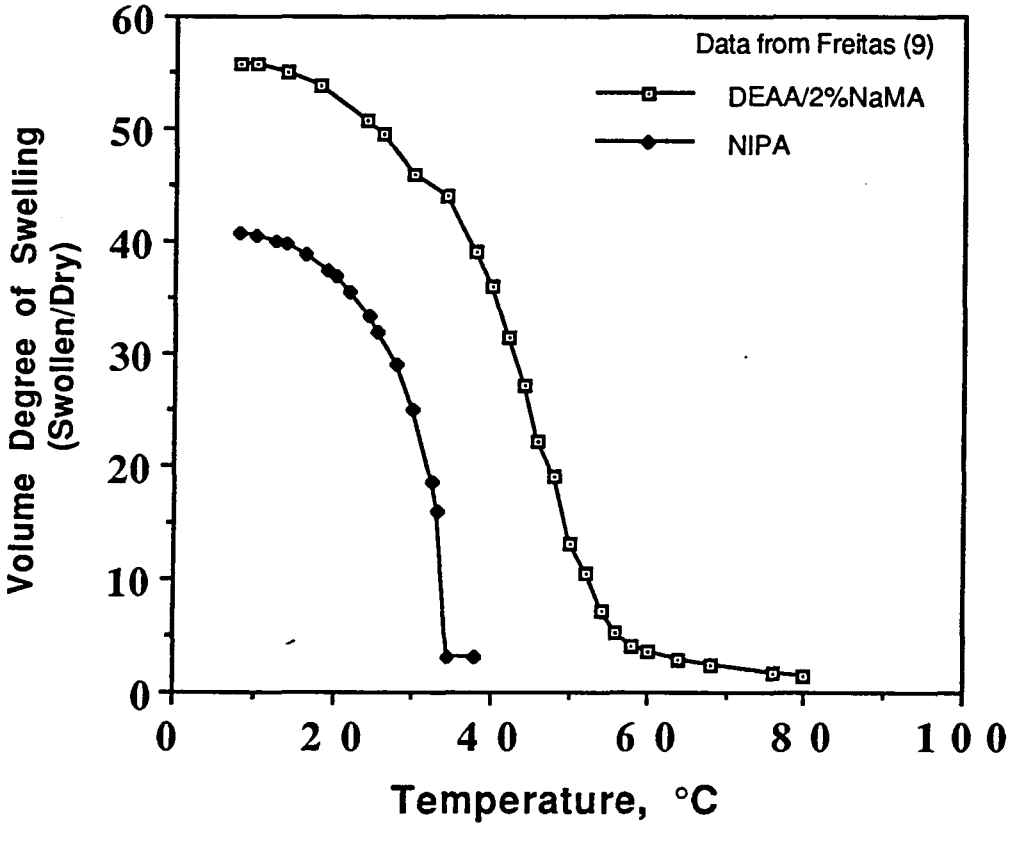


Figure 9

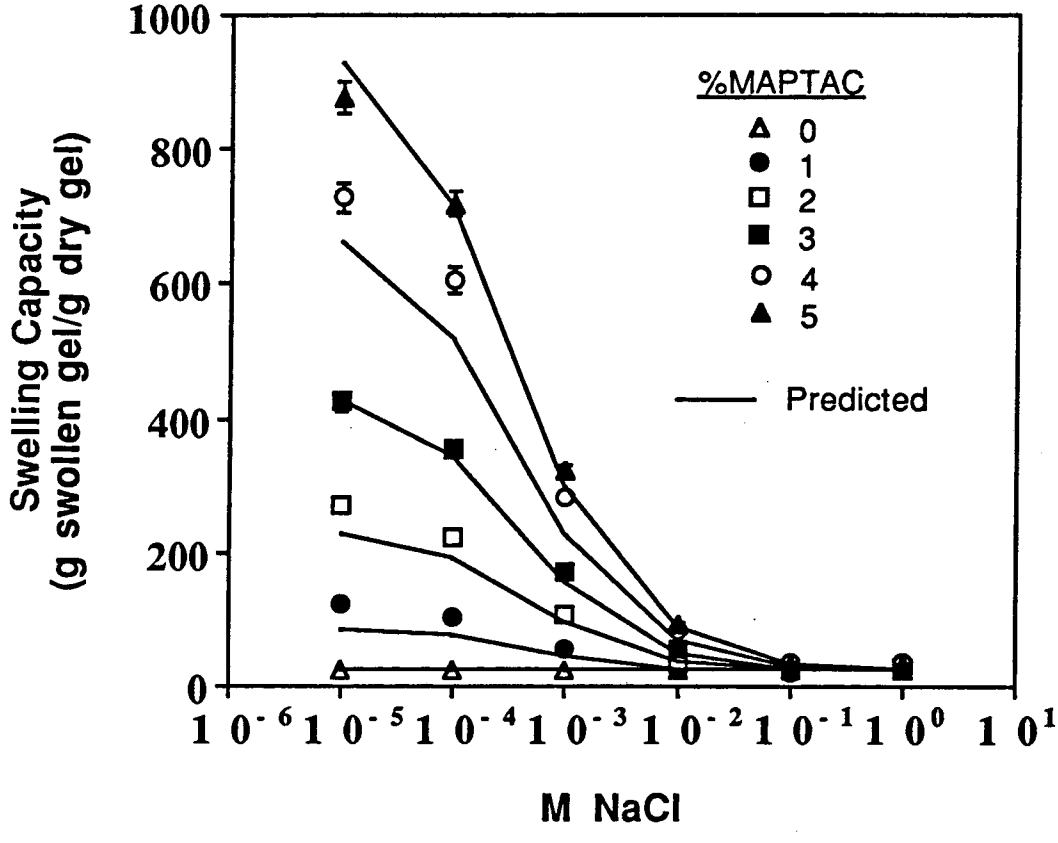


Figure 10

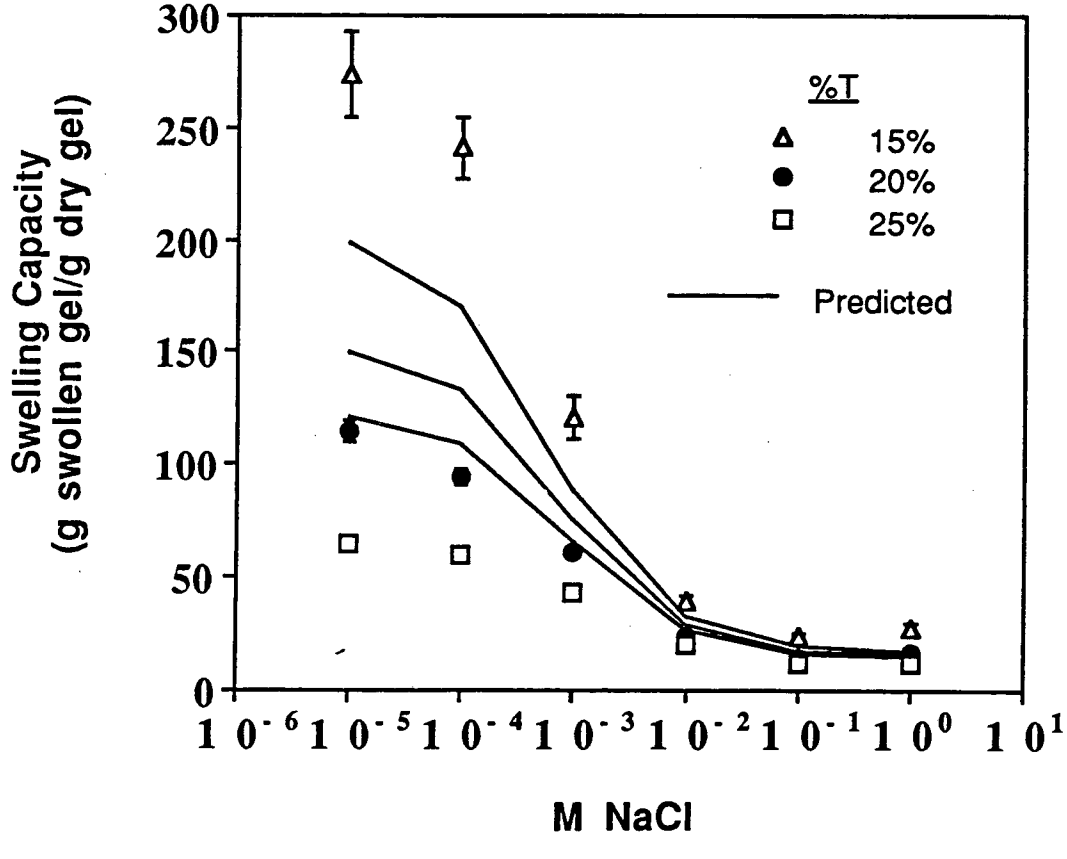


Figure 11

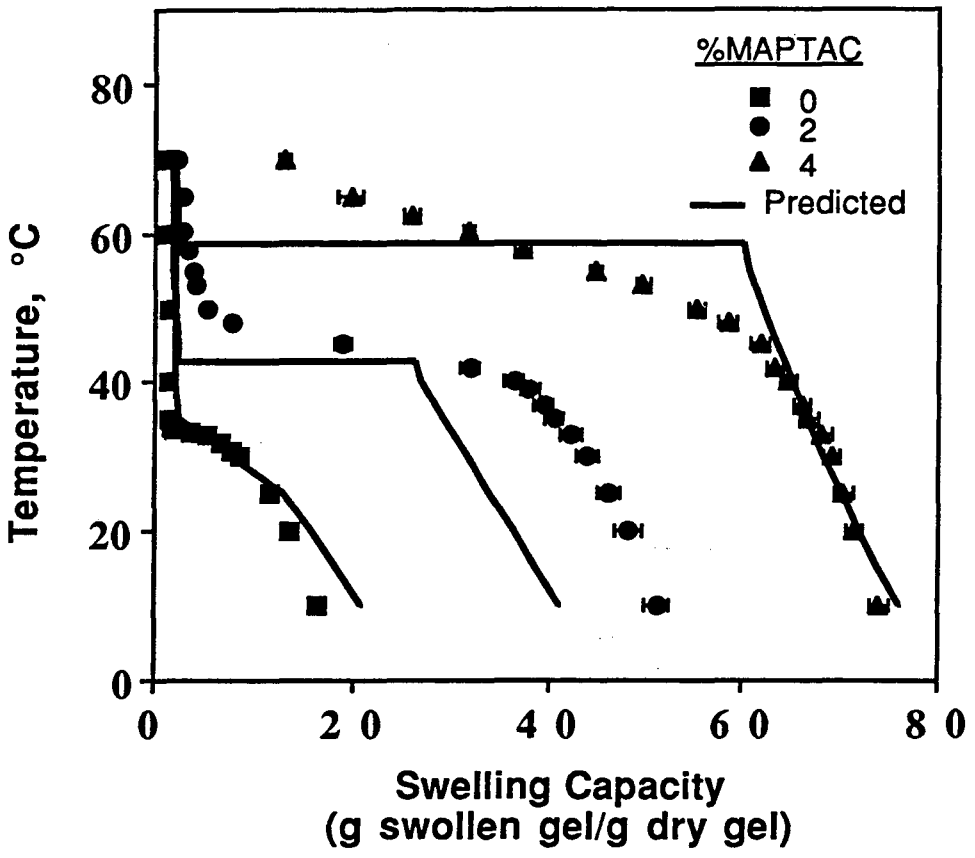
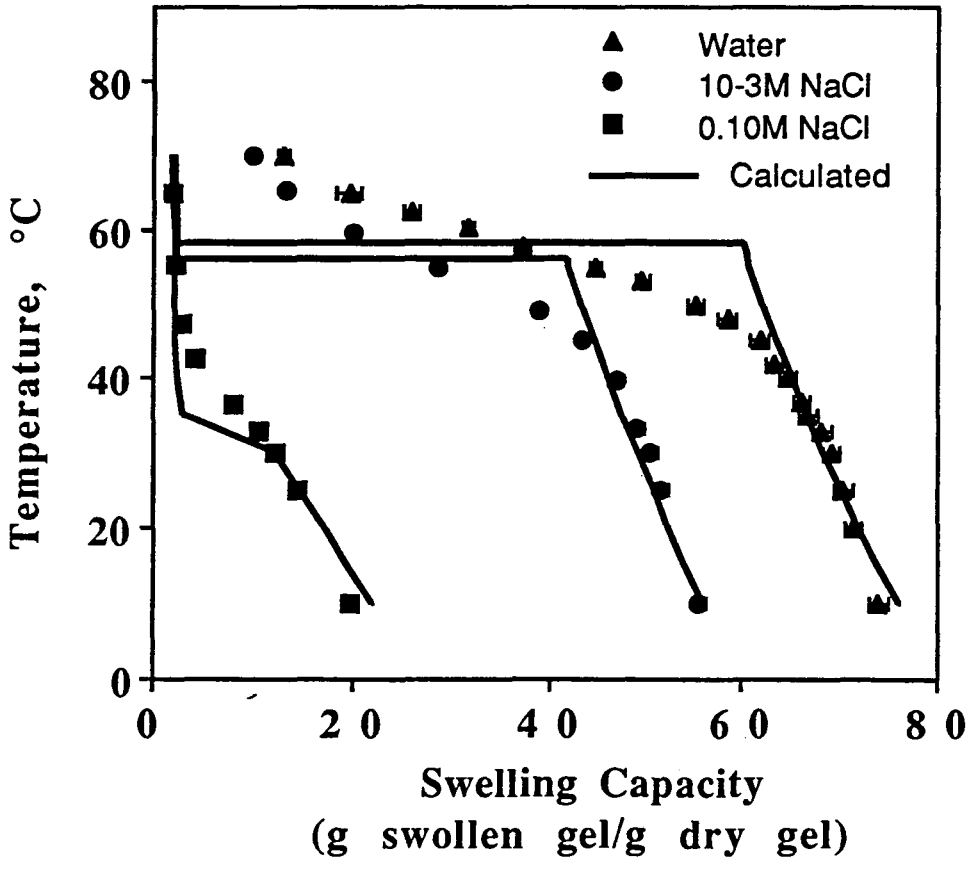


Figure 12



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