Poly(*N*-vinyl-2-pyrrolidone)-polyacrylamide Hydrogels as Extraction Solvents

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Received 23 February 1999; accepted 8 December 1999

ABSTRACT

Polymeric hydrogels, composed of poly(*N*-vinyl-2-pyrrolidone) and cross-linked polyacrylamide have been synthesized for the purpose of concentrating protein solutions by excluding water from them. The hydrogles showed pH-independent swelling behaviour, which was attributed to the non-ionic nature of polymers present in the gel. The hydrogels proved to be an efficient device to extract water from the protein solutions. The extraction efficiency of the hydrogels was found to increase with the increase in cross-linking ratio. Similarly, the gel efficiency has been found to decrease with the increase in concentration of protein solutions. Hence, gels have proved to be an effective device to exclude water from dilute protein solutions. The swollen gels returned to their original shape, when dried in a vacuum oven at 40 °C for 48 h. The de-swollen gels were re-used for the extraction purpose. In order to reflect the quantitative aspect of swelling behaviour, various parameters such as swelling exponent n, diffusion coefficient D, have also been evaluated. The gels with cross-linking ratio 1.5 (in mole%) were found to follow non-fickian swelling mechanism, while gels with cross-linking ratio 3.0 and 4.5 (in mole%) showed Fickian swelling behoviour.

Key Words: poly(*N*-vinyl-2-pyrrolidone), polyacrylamide, extraction solvent, cross-linking ratio, gel efficiency

INTRODUCTION

Hydrogels are unique type of implant polymers. They derive their name from their affinity for water and the incorporation of water into their structures. In recent years hydrogels have received much attention for use as soft contact lenses, [1] burn dressings [2], controlled drug delivery systems [3, 4], immobilized enzyme reactors [5] and separation processes [6].

A hydrogel may be described as a polymeric material that can absorb a significant amount of water (>20% of its dry weight) while maintaining its structural integrity. For applications such as regenerable extraction solvents and soft contact lenses it is important that the three dimensional structure of the hydrogels be conserved. An elastic, not viscous, response to applied stress and the maintenance of mechanical integrity are essential characteristics of

hydrogels [7].

The present paper describes the extraction of water from solutions of macromolecules such as proteins using poly(*N*-vinyl2-pyrrolidone)-polyacrylamide hydrogels which can undergo several swelling/de-swelling cycles while maintaining structural integrity which is the basic requirement for using hydrogels as extraction solvents.

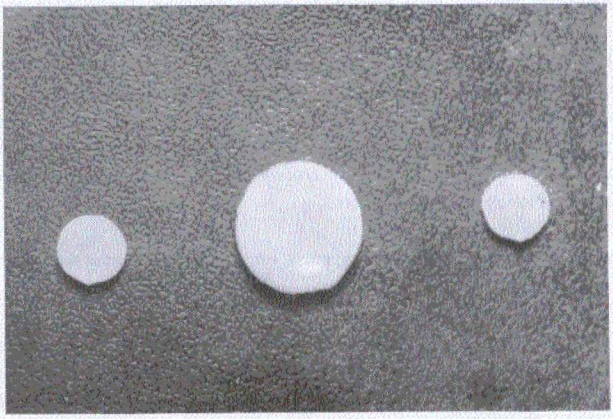
Figure 1 shows different states of similar poly (*N*-vinyl-2pyrrolidone)-(PVP)-polyacrylamide (PAAm) hydrogels. The photograph on the left shows the gel in the completely dried state while the picture in the middle shows the swollen state of gel when put in doubly distilled water at pH 7.0 at 27 °C for a period of 18 h. The photograph on the right is the hydrogel in the de-swollen state, which is attained by putting the swollen gel in a vacuum oven at 40 °C for 48 h. This swelling/de-swelling can be used as the basis of the separation process, which has been schematically described in Figure 2. The basic idea of extraction process is that when the dried gel is put in protein solution, it swells by absorbing water and other small solutes. Because of cross-linked structure of hydrogel it does not absorb macromolecules like proteins. The excluded protein is now in a concentrated solution and is a product of the separation process.

The commercial value of this separation process depends on the regeneration and recycling of the gel. After all, the extraction of water could be done by silica gel. But in order to dry the silica gel for reuse, a high temperature is required. In contrast these gels return to their original shape and size by a slight warming. Such separations have already been carried out before [8, 9], but they were done with non-reusable gels, and hence they could not be exploited commercially. However, the proposed hydrogels can be used on commercial basis on the ground of their easy handling, regeneration at minimum cost and non-toxic nature.

EXPERIMENTAL

Materials

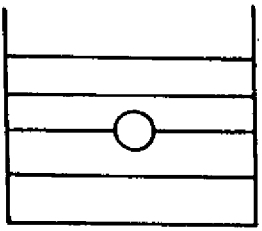
The raw materials used have been described in Table 1. Acrylamide and *N,N'*-methylene bisacrylamide were



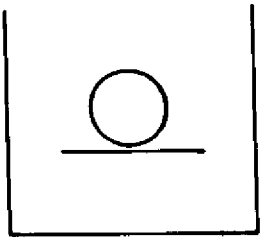
(a) (b) (c)

(a) Hydrogel in the completely dried state; (b) Hydrogel when equilibrated in water at pH 7.0; (c) Gel in re-dried state.

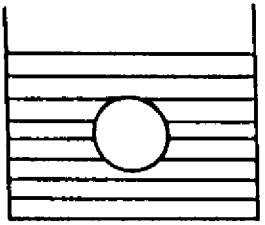
Figure 1. Photograph showing different states of similar PVP-PAAmX (4.5%) hydrogels.



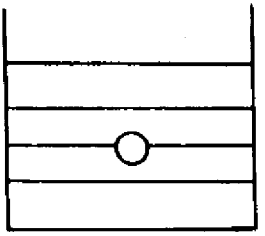
Completely dried hydrogel is put in the protein solution.



The gel is removed from the solution and put in oven for 48 h at 40 °C. The solution is analyzed colorimetrically.



Hydrogel swells by absorbing water, but excluding large protein molecules. The solution becomes concentrated.



The gel returns to its original size and shape, and is ready to be re-used.

Figure 2. Scheme showing extraction of water from protein solution by hydrogel.

recrystallized in methanol before use. Other reagents used were also analytical grade. The double distilled water was used throughout the experiments.

Synthesis of Hydrogels

The initial step is the dissolution of a definite quantity poly(*N*-vinyl-2-pyrrolidone) (3.0 g) in water followed by addition of calculated quantities of monomer acrylamide (4.0 g) and *N,N'*-methylene bisacrylamide (cross-linker). The mixture was stirred gently to give a clear solution. Now to above solution, definite amount of potassium persulphate (0.2 g) was added as initiator and the mixture was stirred until well-mixed and then poured over a mercury pool and left undisturbed for 1 h in an incubator at 70 °C. The hydrogels, so prepared were carefully dislodged from the surface of the mercury pool. Identical sizes of gel discs were made by punching the gel sheet with a cork borer of 3.3 cm diameter, and were dried in a vacuum oven at room temperature. In all, three types of hydrogels with varying cross-linking ratio (in mole%) were synthesized for the proposed study. Here the cross-linking ratio X

Table 1. Raw materials employed and their source.

Case No.	Name and description	Source
1	Acrylamide (AAm)	Robert Johnson[a]
2	*N,N'*-Methylene bisacrylamide	Central Drug House[a]
3	Haemoglobin powder	Loba Chemie Industries[a]
4	Gelatin	Loba Chemie Industries[a]
5	Bovine serum albumin	Central Drug House[a]
6	Poly(*N*-vinyl-2-pyrrolidone) (PVP)	Encore Chemicals[a]
7	Potassium persulphate	Loba Chemie Industries[a]

(a) India

(in mole%) may be given as,

$$X = \frac{\text{No. of moles of cross - linker}}{\text{No. of moles of monomer}} \times 100$$

The three samples will be denoted as PVP-PAAmX (1.5), PVP-PAAmX (3.0) and PVP-PAAmX (4.5) where number in parentheses denotes the cross-linking ratio in mole%.

Measurement of Swelling Ratio

The dried gels were immersed in an excess amount of doubly distilled water at 27 °C until swelling equilibrium was attained, which required almost 18 h. Each sample was then removed from water bath and tapped with filter paper to remove excess surface water and weighed as the swollen weight (W_s). Dry weight was determined after drawing the gel in vaccum oven for 2 days. The swelling ratio Q was calculated from the following formula:

$$Q = \frac{W_s - W_d}{W_d} \quad (1)$$

Dynamic Swelling

The completely dried and pre-weighed gels were placed in excess amount of distilled water and the swelling ratio was obtained by weighing the initial and swollen samples at various time intervals. The amount of water sorbed M_t was reported as a function of time and the equibrium sorption was designated as M_∞. The following equation can be used to calculate the diffusion coefficient D for $M_t/M_\infty \leq 0.85$ [10].

$$\frac{M_t}{M_\infty} = \frac{4}{\sqrt{\pi}} \left(\frac{D.t}{L^2}\right)^{1/2} \quad (2)$$

Where, t is the time and L is the initial thickness of dried gel.

In order to study the effect of pH on the swelling behaviour of hydrogels, two pre-weighed, almost identical, gel discs were put in solutions of varying pH and were allowed to equilibrate. The swelling capacity of gels was calculated.

The reversibility of the swelling process was tested by placing the gel in water at pH 7.0. After attainment of equilibrium the gel was allowed to de-swell by putting in a vacuum oven for 48 h and when it returned to its initial state, it was placed in water again to attain equilibrium swelling.

This process was repeated so many times. The gels showed good swelling/de-swelling capacity without deformation in shape.

Gel Selectivity

In order to study the extraction efficiency of hydrogels, two pieces, almost identical, completely dried and of known weight and dimensions were placed in 20 mL of protein solutions (gelatin, haemoglobin and bovine serum albumin) of known concentrations at 27 °C. The gel-solution mixture was stirred gently on a wrist action flask shaker for 4 h. The swollen gels were taken out of the solution and wiped with filter paper and then weighed accurately. The volume of the swollen gels was determined by measuring their dimensions. The concentration of protein solutions was determined spectrophotometrically using biurate method [11]. The swollen gels were then collapsed by putting in a vacuum oven at 40 °C for 48 h and then they were re-used.

RESULTS AND DISCUSSION

Swelling Dynamics

Typical swelling ratios as a function of time for three gel sample at 27 °C in distilled water have been shown in Figure 3. It is clear from the figure that hydrogel with 1.5 mole% cross-linking ratio shows maximum equilibrium swelling while the sample with 4.5 mole% cross-linking ratio swells to minimum. This is due to the fact that in a highly cross-linked gel, the tight network structure does not allow much water to enter into the gel phase.

Swelling kinetics can be generally described in two terms: the diffusion rate of imbibing solvent into the gel, and the relaxation rate of the polymer network. To obtain a more quantitative understanding of the nature of the sorption kinetics, the initial swelling data were fitted to the exponential heuristic equation [12].

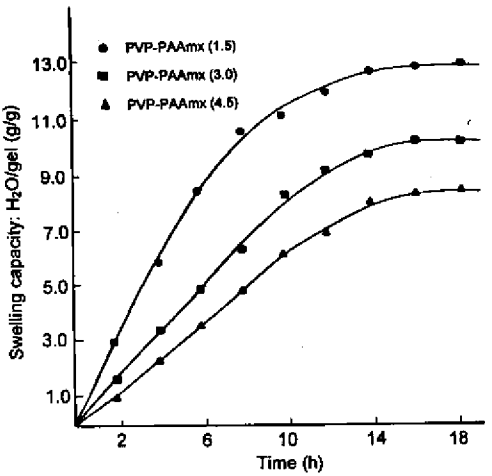


Figure 3. Time vs. swelling capacity for three types of PVP-PAAmX hydrogels at 27 °C and pH 7.0 in water.

$$\frac{M_t}{M_\infty} = kt^n \qquad (3)$$

Where, k is a characteristic constant of the gel, and n is a characteristic exponent of the mode transport of the penetrant, "n" and "k" were calculated from the slope and intercepts of the plot of log M_t/M_∞ against log t. For Fickian kinetics in which the rate of penetrant diffusion is rate limiting, n = 0.5, whereas values of n between 0.5 and 1 indicate the contribution of non-Fickian processes such as relaxation of polymeric chains. The results are shown in the Table 2 which shows that sample PVP-PAAmX (3.0) and PVP-PAAmX (4.5) show Fickian swelling behaviour while sample PVP-PAAmX (1.5) shows non-Fickian behaviour, which may be attributed to the fact that as the cross-linking ratio decreases to 1.3 mole%, the amount of cross-linker in the gel reduces, thus causing a decrease in number of cross-links per unit volume which ultimately results into the availability of greater free volume for the accommodation of solvent into the gel phase.

The measurement techniques of diffusion coefficient in polymer have been fully discussed by Crank and Park [13]. The measurement technique used in their experiment is based on the use of initial rates of sorption It is possible to deduce an average diffusion coefficient from the initial gradient of the sorption curve when plotted against the square root of time.

Eqn (2) was used to calculate the diffusion coefficients (D), which have been shown in the Table 2.

Table 2. Swelling parameters of PVP- PAAm hydrogels at 27 °C at pH 7.0 over a period of 18 h.

Sample	Diffusion coefficient[b] $Dx10^7$ (cm^2/s)	n	Equilibrium swelling capacity[b] (g H_2O/ggel)	k
PVP-PAAmX (1.5)	18.63	0.59	13.0264	0.38
PVP-PAAmX (3.0)	11.05	0.50	10.6291	0.31
PVP-PAAm (4.5)	08.31	0.44	8.5317	0.27

(a) The number in parentheses after the gel sample indicates the cross-linking ratio (in mole%); (b) Average weight of two identical gels taken together.

pH Effect

When hydrogel samples were placed in solutions of varying pH, the swelling behaviour of hydrogels was found to remain unaffected. So pH of the external solution does not influence the swelling capacity of the three hydrogel samples. This may be explained by the fact that the two constituents of hydrogels are poly(*N*-vinyl-2pyrrolidone) and polyacrylamide, which are non-polyelectrolytes. Hence they do not contain any ionizable groups, which may be ionized to produce electrical charges along the polymeric chains. Therefore the possibility of existence of electrostatic interactions inside the gel phase cannot be worked out. Thus swelling remains almost unaffected (see Table 3). Moreover, due to the absence of ioniz-able groups inside the gel phase, the osmotic swelling pressure π_{ion} (which is dominant in polyelectrolyte gels) is not so effective in the present case thus resulting in almost the same extent of swelling in the pH-range studied. Here it is worth mentioning that the

Table 3. Equilibrium swelling capacities of hydrogel samples for different pH of the solution at 27 °C.

pH	Swelling capacity[a]: water/gel (g/g)		
	PVP-PAAmX (1.5)	PVP-PAAmX (3.0)	PVP-PAAmX (4.5)
4.0	12.98	10.59	8.50
7.0	13.02	10.62	8.53
9.2	13.04	10.63	8.51

(a) Average swelling capacity of two identical gels taken together.

author of this paper has also reported the swelling behaviour of haemoglobin-cross-linked polyacrylamide hydrogels [15], which was affected greatly by the pH of the external solution. The effect was explained on the basis of possible hydrolysis of amide groups into carboxylic groups at physiological temperature 37 °C in acidic as well as alkaline medium.

However, in the present case, as the swelling measurements are made at 27 °C, the possible hydrolysis of amide groups of polyacrylamide into carboxylic group is not feasible. In order to justify this argument, the hydrogels of polyacrylamide were synthesized and allowed to equilibrate in swelling media of different pH at 27 °C. The equilibrium swelling capacity was found to be the same for all pH values, thus suggesting that no such hydrolysis had taken place in the polymer matrix.

Reversibility of Swelling Process

Figure 4 shows the continuous swelling and de-swelling of PVP-PAAmX (3.0) gels in double distilled at pH 7.0. It is clear from the figure that the gel undergoes a number of successive swelling/de-swelling cycles without undergoing any deformation in its shape. This shows that gel does not undergo any irreversible structural change during the swelling process. Similar observations have also been reported with hyaluronic acid gel [16]. Indeed, this behaviour of hydrogel forms the basis for its use as extraction solvents.

Extraction of Water

The efficiency of hydrogels to extract water from macromolecular solutions can be expressed by the formula:

$$\eta = \frac{\text{Measured conc. change in protein solution}}{\text{Conc. expected from gel vol. change}} \times 100$$

For example, suppose a hydrogel with known dimensions (and hence volume) is placed in 20 mL of a protein solution of known concentration and owing to the diffusion of solvent into the gel phase, the volume of hydrogel increases by say, 10 mL. It means that the volume of external solution must decrease by the same amount. In other words the volume of protein solution is reduced by 10 mL. As the volume of solution has become half the original volume, the concentration of protein in the solution is expected to increase by a factor of 2. However, if the experimental value shows an increase in concentration by a factor say 1.8 only, then the extraction efficiency will be given as 1.8/2×100 i.e., 90%.

Table 4 shows the extraction efficiency of three types of hydrogels that differ in the mole percent of cross-linking ratio shown in parentheses. These efficiencies in duplicate are accurate within a limit of

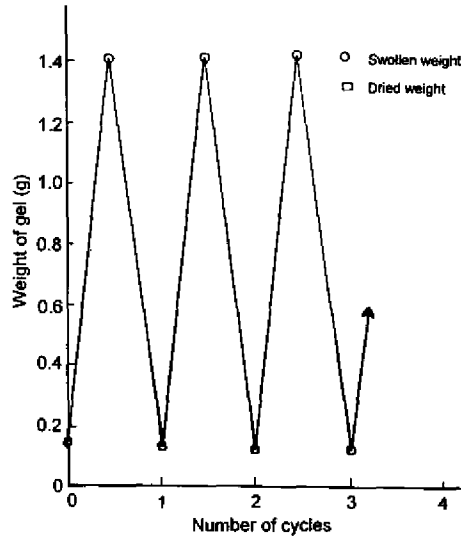


Figure 4. Reversible swelling of PVP-PAAmX (3.0) hydrogel in water; Temperature=27 °C.

Table 4. Extraction efficiency of hydrogels in protein solutions at temperature=27 °C.

Solutions	Gel efficiency[a]		
	PVP-PAAmX (1.5)	PVP-PAAmX (3.0)	PVP-PAAmX (4.5)
Potassium dichromate[b]	–	–	9
Potassium permanganate[b]	–	–	13
Gelatin[c]	83	86	90
Haemoglobin[c]	86	91	93
Bovine serum albumins	84	88	94

(a) All efficiencies were measured in duplicate and the average being reported in table; (b) Concentration is 0.1%; (c) Concentration is 1.0%.

± 2%. The swollen gels were regenerated at 40 °C. It is clear from the Table 4 that these gels absorb solutes of small sizes such as $K_2Cr_2O_7$, $KMnO_4$ but macromolecules like gelatin, haemoglobin, bovine serum albumin are excluded due to large dimension of their molecules. It is also clear from the Table 4 that as the cross-linking ratio increases; the extraction efficiency of gel also increases. Therefore, the gel behaves like a filter that excludes large molecules. If this prediction is correct then the gel efficiency should increase with mole percentage of cross-linking ratio. In order to verify this, the gels of varying percent of cross-linking ratio were placed in solutions of gelatin and their extraction efficiency was determined (Figure 5). It is clear from the Figure 5 that as the cross-linking ratio in hydrogel increases, the extraction efficiency also increases. This means that the use of a more highly cross-linked gel may allow separation of smaller sized solute molecules provided that other possibilities are not interfering.

However, a gel does not suddenly become effective, it does not show an abrupt molecular cut off. It does not suddenly jump from 0% to 100%. This is consistent with a distribution of cross-links typical for random polymers.

From the above discussion one point is clear that the gels selective absorption depends on solute size. For example, as indicated in Table 4 the hydrogels absorb $K_2Cr_2O_7$ and $KMnO_4$ but they exclude protein solutions containing macromolecules. This exclusion concentrates the solution gently without protein denaturation. This is because the process does not significantly change the pH or the ionic strength, nor does it subject the solutes to high pressure or high shear. Similar experiments with pH-sensitive gels show that micro-organisms can be concentrated while remaining viable. Here it is worth mentioning that when hydrogels, swollen in protein solutions, were dried completely and weighed, their weight was slightly more (0.2–0.5 mg) than their initial dry weight (0.2700–0.2900 g). This could have been due to possible adsorption of protein molecules on the hydrogel surface. This also explains that why gel efficiency was not found to be 100%. However, owing to the negligible extent of adsorption, the studies were not concentrated on this aspect.

One more reason for not achieving 100% efficiency is that the polymeric chains are randomly distributed in the polymer matrix and hence during the swelling of gel, some protein molecules may have entered into the gel phase through the sites where cross-linking is relatively less to provide sufficient space through which macromolecules may have an opportunity to enter into the gel phase.

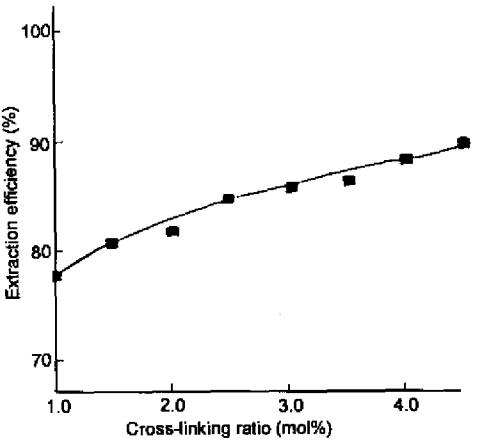


Figure 5. The effect of variation in percent cross-linking on the extraction efficiency of PVP-PAAmX hydrogels in gelatin solution. Temperature=27 °C, concentration of gelatin=1%.

As a final test of selectivity, the effect of concentration of feed solution on the efficiency of the hydrogels have been depicted in Figure 6 which clearly shows that the concentration of feed solution increases, the efficiency of gel decreases. Hence for dilute solution of macromolecules this is an excellent method to exclude water. However, as the concentration of feed solution increases, the separation process is not being so effective. For a feed concentration of 4% of gelatin, the separation is only 55% efficient. Entrainment between gel particles can compromise the efficiency of separation. In particular, the data in Figure 6 shows that the efficiency of water exclusion is high in dilute solutions, but significantly low for more concentrated feeds. The possible reason for less efficient exclusion at higher concentrations may be due to small amounts of raffinate being trapped between the particles of swollen gel. This entrained raffinate contains high concentrations of solute. This entrainment may be reduced by washing or by more complete filtration of the gel. Moreover it should also be noticed that the higher concentrations of protein solution might decrease the extent of swelling of hydrogel, thus finally reducing the gel activity.

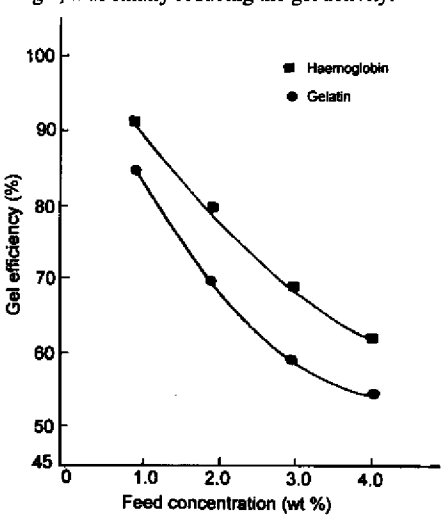


Figure 6. The effect of variation in feed concentration of gelatin and haemoglobin solutions on the extraction efficiency of PVP-PAAmX (3.0) hydrogels, temperature=27 °C.

The author of this paper has, however, not made any attempt to see whether these gels show any chemical selectivity. For example, it is not certain if they can separate ethanol and water (azeotropic mixture). In a preliminary experiment, the pre-weighed hydrogel was put into dehydrated ethanol for a period of 3 h, but the hydrogel did not show any sign of swelling and its weight remained constant, thus suggesting that it had no affinity for ethanol towards swelling. In another set of experiments the gel was placed in a mixture of water and ethanol (containing 20% water v/v), for a period of 2 h and it was found that gel showed some swelling (10% of its dry weight). Now the question is that whether the swollen gel contains pure water or the mixture of two components? Moreover, in a highly swollen gel the amount of water is so large that the free energy of ethanol molecule may be almost the same as that in the external solutions [17]. Therefore, it is suggested that the chemical separations using highly cross-linked or less swollen gel is a new field in which a wide range of possibilities lie for further work.

CONCLUSION

Thus it is concluded from the above study that PVP-PAAm hydrogels can be used to extract water from protein solutions without causing their denaturation. The efficiency of separation depends upon solute size and degree of cross-linking. These gels can be easily regenerated by keeping them at 40 °C for a period of 48 h. In brief, this type of separation represents a low cost alternative to ultra-filtration. The proposed separation process may also be used for purification of protein solutions.

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