Supersorbent Gels for the Separation of Proteins from Dilute Aqueous Solution

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Summary: We have successfully used size selective P(N-Isopropyl acryl amide-co-Acrylic acid) gels to concentrate dilute aqueous solutions of high molecular weight Bovine serum albumin, dextran and poly vinyl pyrrolidone. Concentration factor of 2.4 with 96% efficiency was obtained for dilute solution while for semi dilute solution it was 1.77 with 81% efficiency. Desired pore size of the gel is obtained by changing the concentration of monomer or cross linker while changed LCST is possible by modifying copolymer composition / change in pH of the aqueous environment. The resultant inexpensive and feasible under mild conditions separation process is alternative to ultrafilteration.

Introduction

Life is polymeric because the most important components of living cell (proteins, carbohydrates etc) are all polymers and nature uses it both for the construction and as part of the complicated cell machinery of living organism. Protein and metabolites when concentrated from biological sources, involve a number of purification steps like precipitation, vacuum dialysis, foam separation, freeze drying and so many others [1-4]. But these general techniques are rarely suitable for separation of delicate biological products that are typically produced in dilute solution having several contaminants. Moreover these separation/ clean up methods involve considerable amount of organic

solvents that generate serious problem with respect to human health and ecological systems. Cross linked hydrophilic polymers can be used as supersorbent [5] which are capable of absorbing water in excess and small solute leaving proteins like big molecules. In the past many polymers have been used for the extraction purposes [6,7] but with limited success due to: 1) less swelling capacity of polymer and more expensive 2) not complete exclusion of large molecule and disturbance of biological activity of solute and 3) instability over repeated cycles of swelling and collapse. Moreover, the removal of water from starch and cheese, the concentration of antibiotics in fermentation beers and recovery of protein products of genetically engineered microorganisms need attention to be addressed. This paper, third of the series, of hydrophilic P(NIPA-co-AA) explores new separation methods for these systems.

Results and Discussion

We have studied the variation of swelling ratio 'r' with temperature for P (NIPA-co-AA) hydrogel at four different pH i.e. 2, 6.2, 8, and 12 and also for the whole pH range (1-14) and the results are shown graphically in fig 1 and 2 respectively. Generally at any temperature smaller than lower critical temperature (Tc), irrespective of polymer composition and cross linker contents, the swelling was enhanced by the acidic co monomer, being more than MAA swelling ratio [8]. The co monomer AA is a weak acid [9] and at pH 6.2 its dissociation is quite low < 0.3 and the acidic unit is in protonated form and at high pH in ionised form. The swelling ratio increases with pH up to 9.7 and then decreases on further increase of pH. Simple explanation for this is that with increasing pH, AA on the network chain is ionised, resulting in swelling due to osmotic power of the counter ions [11] and beyond pH 9.7 the added NaOH behaves as an added salt screening ionic interaction and the gel behave as it was uncharged. We made two solutions having pH 12 and 2 and by addition of HCl / NaOH adjusted the pH and determined the swelling ratio. Interestingly the 'r' for pH up process is less than pH down process, which is unexplainable.

At low pH, the lack of repulsive interaction, rather occurrence of hydrogen bonding [11-13] among the carboxylic groups and with the amide groups of NIPA unit, make the hydrogel more compact and minimized the swellability (reduced

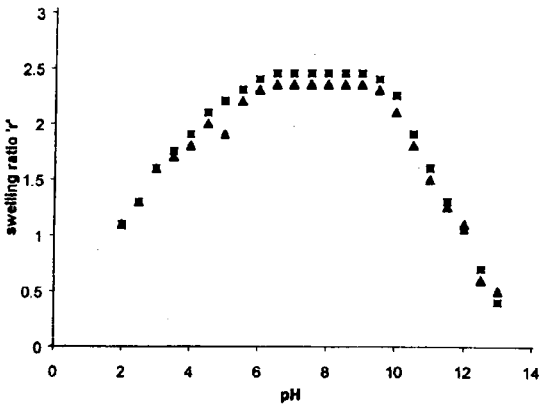

Fig-1: Swelling ratio 'r' variation of P(NIPA-co-AA) gel with pH at 23°C (■) = pH-down. (▼)=pH- up

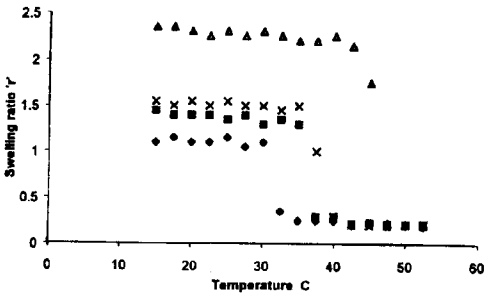

Fig-2: Swelling ratio variation with temperature at different pH. ♦--pH = 2, ■--pH = 6.2, ∇ -- pH = 8, ×--pH = 12.

interaction with water). These hydrophobic interactions, due to protonation of acidic units and more compact conformation of the hydrogel, are responsible for lower value of critical temperature i.e. 32°C. Beltran *et.al.* [14] Observed that for copolymers of higher content of AA and pH, the deswelling is continuous and no Tc is detected within the experimental temperature range. However in our case, though not very prominent still it appears that Tc is near 40°C. Although high AA contents give rise to enhanced hydrophillicity but at pH 12 free negatively charged AA units cannot be ignored. The result of the present study, need comparison with the temperature and pH dependent behaviour of hydrogel [15] and copolymers [16] constituted of the same material with different ratios. Hydrogels composed of < 14mol % AA, were claimed [17] to have

temperature sensitive conformational changes only over a limited pH range of 4-7 but here the situation is quite different. In fact such system gave cloud points leading to phase separation, over both pH (3—9.7) and temperature (20—40°C) range, showing that LCST can be altered by slight modification of copolymer composition or changes in pH of the aqueous environment.

Pore Size

The exclusion behaviour of the copolymer gels was studied and reported [18] in term of distribution coefficient Kd, which is the ratio of solute concentration in the gel phase, to that in the retentate, C_R:

$$K_d = C / C_R$$

When Kd is zero complete exclusion occurs and when Kd is ∝ complete removal from solution. With changing pore size of the gel, the exclusion behaviour also changed. Gels with smaller pore sizes of the network were prepared by increasing the concentration of monomer or of the cross linking agent at gel formation [19] so that the model protein molecules may not penetrate in to the P (NIPA-co-AA) gel. Our previous findings showed that PVP with molecular weight above 25,000 did not penetrate in to gel so we selected the model protein above this. Beside pore size and solute size, the shape of solute molecule is also important. It was observed [2] that an extended molecule of low molecular weight was completely excluded while a globular enzyme like lipase of Mw $=10^5$ were partitioned with a distribution coefficient of 0.2.

Concentration of BSA, Dextran and PVP

The success of the proposed separation/ concentration process is heavily dependent on: firstly that the gel absorption is selective, for the gels absorb the low molecular weight solvent but not higher molecular weight solutes, secondly how the gel can be regenerated and reused; this regeneration exploits the large changes of gel volume with small variation in pH and thirdly that the gel are sufficiently strong to be used repeatedly. Table –1 shows the molecular weight, solute size, feed concentration, and raffinate concentration of sample, volume ratio and efficiency of the gel. Gel selectivity was discussed before in pore size while the effectiveness of the protein separation can be quantified by an efficiency η,

Table-1: Concentration of dilute solution of BSA, PVP and dextran using P(NIPA-co-AA) gels.

Solute	M.wt	Size (nm)	Feed wt %	Raffinate conc.%	C.F.	Vo/V	Efficiency
BSA	60,000	6.9	0.100	0.243	2.430	2.530	96
			0.150	0.304	2.030	2.200	92
			0.200	0.397	1.985	2.200	90
			0.250	0.471	1.884	2.190	85
			0.500	0.885	1.770	2.180	81
PVP	40,000	4.7	0.150	0.315	2.100	2.170	97
			0.250	0.472	1.888	2.020	93
			0.500	0.892	1.784	2.070	86
Dextran	71500	5.3	0.250	0.435	1.741	1.850	94
			0.500	0.812	1.624	1.840	88

which is actual increase in protein concentration divided by the increase expected from the altered solution volume [6], that is

η = 100 (measured conc. Change / expected from gel volume change)

or

$$\eta = 100\,(C-C_o) \;/\; [C_o(V_o / V) - 1)]$$

For example if half of the feed solution is absorbed by the gel ($V_o/V_{=2}$), and if the raffinate has twice the feed concentration then the efficiency would be 100%. If the raffinate concentration is equal to the feed one then efficiency is 0 % and if the concentration increases by a factor of 1.8, then the efficiency is 90%. These efficiencies (in the last column of the table and measured in triplicate) are accurate within 4.3%. All extractions were carried out at 23°C and P (NIPA-co-AA) gel was regenerated at 40°C. The results show that in the range of protein concentration tested, higher concentration factors and recoveries are obtained with more dilute solutions. Concentration factor up to 2 together with high recoveries (> 96 %) were obtained using small amount of polymer. This is further supported by the findings that a decrease in the recovery of proteins, the concentrated becomes the final solution. This is attributed to increased entrainment of more concentrated solutions due to increased viscosity.

The less efficiency at higher concentration of BSA, PVP and dextran is justified due to its weak adsorption on the surface of the gel spheres. In one case of 0.5 wt% feed concentration of BSA when we washed the gel, the extraction efficiency enhanced to 86 from 81%. The present concentration factor and recoveries can be compared with other researchers for the concentration of BSA but with different polymers. Vashaghani [2] observed a loss of efficiency in the concentration process of ovalbumin

with P (NIPA-co-sod. acryl ate) gel when the protein concentration was increased. Badiger *et.al* [4] reported concentration factor of 4.6% and 98 % recovery for poly (styrene-sulfonic acid) gel used in a column. Although in the referred work performance is better but information's concerning the amount of polymer used, initial concentration, ionic strength and pH is lacking.

Experimental

The synthesis of supersorbent P (NIPA-co-AA) copolymer was discussed elsewhere [19] while Bovine serum albumin (BSA), Dextran and PVP from Fluka Biochemika and deionised water were used for the experiments. 0.1352g of xerogel disc M_1 was placed in a glass, appropriate volume V_1 of aqueous solution with pH-6.2 (adjusted with a base or acid) and BSA concentration C_1 was added. After 120 minutes, (because the process practically completed after 120 minutes for all conditions tested) the swollen gel was weighed M_2. The final volume volume V_2 was measured and after centrifuging the sample, the final concentration C_2 of BSA at 280 nm. Actually this is a five steps process: a) Add solution to pre weighed xerogel. Gel swells, preferentially absorbing solvent. b) Withdraw/separate non-absorbed raffinate, now a concentrated solution. c) Recover the swollen gel by centrifugation. d) Add acid to gel due to which it shrink drastically and then withdraw the released solvent and finally e) add base to gel to make it ready for reuse in step-a. All experiments were performed at room temperature i.e. 23 °C.

Swelling ratio 'r' $= M_2 / M_1$

Concentration Factor (C.F.) $= C_2 / C_1$

Recovery (%) $R = 100V_2C_2 / V_1C_1 = C.F\ (100C_f / V_o)$

And the volume of water absorbed by gel was correlated with xerogel swelling rate and xerogel mass by:

$$V_1 - V_2 = 1.27 + 1.146\ M_1\ (r - 1)$$

Where $M_1\ (r-1) = M_2 - M_1$ i.e. xerogel mass increase.

Conclusions

Cross linked polymer gels whose swelling is sensitive to temperature, pH or concentration of surrounding medium, can be exploited to concentrate dilute aqueous solution of macromolecular solutes including proteins in a gel extraction process. Concentration factor of 2 (extendable to 3.5) with 96% recovery were obtained using small amount of polymer. The performance of the process improved considerably when concentrating more dilute solution. This methods/process has some potential for separating high molecular weight proteins present in dilute solutions (antibodies) with low salt content. High molecular weight proteins can be purified from low molecular weight proteins if the later are capable of penetrating the gels and above all this process is inexpensive, feasible under very mild condition with no problem of extreme temperature, pressure pH change or contamination and denaturation due to high shear fields. . LCST can be altered by slight modification of the copolymer composition or change in the PH of the aqueous environment.

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References

1. G. Walsh and D. Headon; Down stream processing of proteins product; John Wiley and sons; Inc. Chichester, PP-39, (1994).
2. V. Farahani, E. Cooper and M.E. Weber, *Chem. Eng. Sci.*, **47**(1), 31 (1992).
3. S.H. Gehrke, G.P. Andrews, and E.L. Cussler, *Chem. Eng. Sci.*, **42**, 97(1987).
4. M.V. Badiger, M.G. Kalkarni, and R.A. Mashelkar, *Chem. Eng. Sci.* **47**, 3 (1992).
5. B.L. Peppas, and N.A. Peppas, *Chem. Eng. Sci.* **46**, 715 (1991).
6. R.F Freitas and E.L Cussler, *Chem. Eng. Sci.* **42**, 197(1987).
7. S.J. Frank, D.W. Johnson, E.L. Cussler, *J.Fd. Technol.*, **43**(6), 79 (1989).
8. X.Wei, S.Champ, M.B.Huglin, *Polymer*, **42**, 2247 (2001).
9. M. Nagasawa, T. Murase, K. Kondo, *J. Phys. Chem.*, **69**, 4005(1965).
10. V. Farahani, E.Cooper, M.E. Weber, *Ind. Eng. Chem. Res.* **29**, 554 (1990).

11. E. Meaurio, T.L Valeda, L.C. Cesteros, I. Katime, *Macromolecule,* **29**, 4598(1996).
12. Y. Wang, H. Morawetz, *Macromolecules*, **22**, 164 (1989).
13. F.O. Garces, K. Sivadasan, N.J. Turro, Macromolecules, **27**, 272 (1994).
14. S. Beltran, J.P. Baker, H.H. Hooper, H.W. Blanch, *Macromolecules*, **24**, 549 (1991).
15. L.C. Long and A.S Hoffman, *J. Controlled Release*, **15**, 141(1991).
16. G. Chen and A.S. Hoffman, *Nature*, **49**, 373 (1995).
17. N.J. Snoden, M.J. Marston and B.Vincet, *Colloid. Polym. Sci.*, **272**, 1273 (1994).
18. S. Bashir, Z. Iqbal and B. Ahmad, *Science International* , (2002) accepted.
19. S. Bashir, Z. Iqbal, B. Ahmad, M.N.Ahmad, S. Zakir. *Jour. Chem. Soc Pak.* (2002) accepted.