ELSEVIER

PII: S0144-8617(97)00243-9

Carbohydrate Polymers **35** (1998) 7–12

0144-8617/98/$19.00+0.00

A preliminary study of the use of larch arabinogalactan in aqueous two-phase systems

Ted J. Christian[a], Merilyn Manley-Harris[a,*], G.N. Richards[b]

[a]*Chemistry Department, University of Waikato, Private Bag 3105, Hamilton, New Zealand*
[b]*The Shafizadeh Center for Wood and Carbohydrate Chemistry, University of Montana, Missoula, MT 59812, USA*

(Received 5 August 1997; revised 4 November 1997; accepted 10 November 1997)

The polysaccharide arabinogalactan (AG) can provide a low cost alternative to the fractionated dextrans for use with polyethylene glycol (PEG) in aqueous two-phase, two-polymer systems. The narrow molecular weight range and low viscosity at high concentration of AG provide for predictable and reproducible separations under a variety of conditions. The partition behavior of bovine serum albumin (BSA) was studied under varying conditions of pH, AG:PEG concentration ratios, and PEG molecular weights. Batch extractive bioconversions of corn starch to cyclodextrin and glucose were demonstrated using AG–PEG systems. © 1998 Elsevier Science Limited. All rights reserved.

INTRODUCTION

Two-polymer aqueous two-phase systems provide a mild and selective method for extraction of biological materials that has found extensive use in the laboratory (Albertsson, 1986). The two polymers most commonly employed are polyethylene glycol and a polysaccharide, usually one of the fractionated dextrans. The techniques used in this type of extraction have the potential to find extensive use in the biotechnology industry. Most industrial-scale aqueous two-phase separations use PEG/salt systems, which may damage fragile proteins (Johansson, 1974) and which present waste disposal problems (Vernau and Kula, 1990). Two-polymer systems can overcome these drawbacks. However, the high cost of the fractionated dextrans has prevented their use on a large scale (Kroner *et al.*, 1984). A number of low-cost alternatives have been investigated, including crude dextran (Kroner *et al.*, 1982), maltodextrins (Szlag and Giuliano, 1988) and hydroxypropyl starch derivatives (Ling *et al.*, 1989; Sturesson *et al.*, 1990). Crude dextran provides a four-fold reduction in cost relative to the fractionated dextrans (Kroner *et al.*, 1982). Systems using crude dextran show high viscosity and subsequent slow separation behavior, although this problem might be overcome in technological applications. Hydroxypropyl starch derivatives display excellent separation behavior; however they effect only an approximately two-fold reduction in cost (Kroner *et al.*, 1982), which is insufficient to make them a viable alternative to fractionated dextrans for industrial scale applications. Maltodextrins can be obtained at a cost that is sufficiently low to make them a viable alternative (Szlag and Giuliano, 1988), but cannot be utilized for extended periods in processes involving starch-degrading enzymes. Furthermore, their relatively low molecular weight may cause osmolality problems at high concentration.

We have now shown that arabinogalactan has potential to act as an alternative to the fractionated dextrans. AG is a water-soluble polysaccharide found in the wood of species of the genus *Larix* and may constitute up to 35% of the total heartwood of some species (Whistler, 1993). Arabinogalactan is available commercially in ultra-filtered grade (AG-UF) and in food grade (AG-FG). Both grades of AG exhibit polydispersity (M_w/M_n) of 1–2, which is comparable with the fractionated dextrans. UF grade AG has an osmolality of 75 mOsm kg^{-1} (at 30%, w/w) with AG contributing about 25 mOsm kg^{-1}. Food grade AG contains a 1–10 mM concentration of non-AG components, primarily salts[1]. The low viscosity (Owens, 1940) and low osmolality of AG solutions at high concentrations and the narrow molecular weight range of the polysaccharide mean that it exhibits relatively

*To whom correspondence should be addressed.

[1]Information supplied by Larex Inc, St. Paul, MN.

rapid and complete separations in aqueous two-phase systems. Partitioning behavior of enzymes and proteins in the AG:PEG two-phase system is similar to that observed in systems employing fractionated dextrans. Additionally, AG is not susceptible to attack by starch-degrading enzymes. Thus, we have been able to demonstrate that it may be used for aqueous two-phase systems in which starch is converted to glucose or to cyclodextrins. Two-polymer aqueous two-phase systems are particularly well suited to the production of cyclodextrins since the hydrophobic interior of cyclodextrin molecules makes them partition favorably to the upper, less polar phase, where they may be more economically removed than if they partitioned to the lower, AG-rich phase.

EXPERIMENTAL

Materials

Arabinogalactan and fractionated dextran (Sigma D-1037) were kindly supplied by Larex Inc. (St. Paul, MN). Polyethylene glycols 4500 and 8000 (PEG 4500, PEG 8000) were obtained from Dow Chemical Company (Midland, MI), and 20 000 (PEG 20M) from Fisher Scientific Company (Pittsburgh, PA). Cyclodextrins (cyclohexaamylose, cycloheptaamylose, and cyclooctaamylose; α-, β-, and γ-CD, respectively), corn starch, and Biuret total protein reagent were purchased from Sigma Chemical Company (St. Louis, MO). Proteins and enzymes used were bovine serum albumin (BSA, fraction V), amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger*, and α-amylase (EC 3.2.1.1) from *Bacillus* sp, all purchased from Sigma. An aqueous solution of Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) from *Bacillus macerans* was kindly supplied by Amano Enzyme USA Co., Ltd. (Troy, VA). The specific activity of the CGTase solution, as stated by the manufacturer, was >600 units g^{-1} as determined by the method of Tilden and Hudson (1942).

Analyses

Cyclodextrins and glucose produced by enzymic conversion of corn starch were quantified by liquid chromatography (LC) using, respectively: Method A, Waters 8 mm × 10 cm μBondapak NH_2 radial compression column, eluted with acetonitrile:water (30:70, v/v) at 2.0 ml min^{-1}; and Method B, Waters 8 mm × 10 cm Resolve C_{18} radial compression column, eluted with water at 1.0 ml min^{-1}. Raffinose·$5H_2O$ served as internal standard for both analyses, and relative response factors were determined previously using authentic compounds. Protein was analyzed by Biuret using BSA as a standard (Doumas *et al.*, 1981).

Phase diagrams

The binodals for AG with PEG 4500, PEG 8000, and PEG 20M were determined by turbidimetric titration (Albertsson, 1986). Tie lines for the AG–PEG 8000 system were determined (Albertsson, 1986) using a combination of polarimetry and freeze drying. The relatively small specific optical rotation of AG is very sensitive to changes in both temperature and PEG concentration. Therefore a temperature-controlled, jacketed cell was employed (at 30 ± 0.5°C) for all optical rotation measurements and the corrected value of $[\alpha]_D^{30}$ was taken from a calibration experiment in which the observed specific rotation for AG was plotted against PEG concentration. Viscosity of phases was measured at 25°C on a Brookfield DV-II digital viscometer.

Partitioning of bovine serum albumin

BSA was dissolved in a variety of buffers and combined with AG and PEG stock solutions. All systems contained 16.0% AG and 6.0% PEG 8000 (w/w, ±0.1%), and ~50 mg (accurately weighed) BSA overall. Each mixture was then gently vortexed for 30 s, equilibrated to 25°C for 30 min, centrifuged 5 min at 2000 × *g*, and re-equilibrated to 25°C for 15 min. Phase volumes were noted and the upper and lower phases of each system were analyzed for protein. Mass balance determinations showed 91 ± 2% recovery of BSA in each case, with no perceptible adsorption to the interface or precipitation of protein. The effects of variations in PEG molecular weight and polymer concentrations were studied using a single buffer system and the same techniques.

Enzymic conversion of starch to cyclodextrins

The two-phase system employed to convert corn starch to α-, β-, and γ-cyclodextrins consisted of 16.0% AG and 6.0% PEG 8000 buffered to pH 6.0 with 0.1 M sodium phosphate. Stock solutions of the polymers in buffer were combined, brought to 20 g total with buffer, mixed by vortexing, and allowed to settle for 30 min at 25°C. After recording phase volumes, 1.0 g corn starch was added and gelatinized by placing the system in a boiling water bath for 30 min, with intermittent stirring. The starch was then liquefied by taking the system to 80°C, adding CGTase solution (25 mg), and stirring continuously. After 1 h the system was immersed in a water bath at 25°C for 15 min, centrifuged at 2000 × *g* for 5 min, and re-immersed at 25°C for 15 min. A 'time zero' sample was taken from the upper phase, an additional 25 mg CGTase added, and the system heated to 60°C, with continuous stirring. The settling and centrifuging treatment at 25°C, with sampling of the upper phase and subsequent reheating to 60°C was repeated at intervals.

Each sample (~100 mg accurately weighed) was immediately diluted with water (1.0 ml containing 1.0 mg raffinose·$5H_2O$). Absolute methanol (0.15 ml) was added, and the samples were thoroughly mixed and passed through a solid-phase extraction cartridge (SPE, Supelclean LC-18 pre-conditioned with 15% methanol in water) eluted with 15% methanol in water to remove the polymers. The eluate

was evaporated to near dryness and re-dissolved in water (0.25 ml) for later LC analysis by Method A. The concentration of each cyclodextrin in the lower phase of the experimental systems was determined using upper phase concentration, partition coefficient, and lower phase volume. Partition coefficients for each cyclodextrin were determined from a similar two-phase system without starch or enzyme. To compare coversion in a single-phase system the conversion was repeated in buffer without AG or PEG. The procedure was identical to that for the two-phase system with the exception that centrifuging and polymer removal by SPE were unnecessary.

Enzymic conversion of starch to glucose

Corn starch (5%, w/w) was hydrolyzed by a mixture of α-amylase (75 units ml^{-1}) and amyloglucosidase (14 units ml^{-1}) at 50°C in 16.0% AG and 6.0% PEG 8000 buffered to pH 5.0 with 0.1 M sodium acetate (Larsson *et al.*, 1988). Samples were analyzed intermittently for glucose by LC Method B. For comparison, the same conversion was carried out in a single-phase buffer solution containing neither PEG nor AG.

RESULTS AND DISCUSSION

Phase diagrams, density and viscosity of phases

Aqueous mixtures of AG and PEG form two phases, an upper PEG-rich phase and a lower AG-rich phase. The phase behavior of these systems is illustrated in Fig. 1. There is no significant difference between the binodals of AG-UF and AG-FG when combined with PEG 8000. The binodal (curved line) represents the lowest combinations of PEG and AG which will produce two phases. A tie line (straight line) represents two-phase systems whose upper and lower phase compositions are identical but that differ only in the relative volumes of upper and lower phase. The points at which the tie-line intersects the curve, X_1 and X_2, represent upper and lower phase compositions, respectively.

As the average molecular weight of PEG increases from 4500 to 20M (Fig. 1*B*), the binodal moves downward and to the left, symmetry increases, and the concentration of polymers needed to form two phases decreases. This is expected since the molecular weight of PEG 20M more closely resembles that of AG (Albertsson, 1986), which is ~37 kDa as determined by light scattering (Prescott *et al.*, 1995). The advantage of using less PEG by increasing its molecular weight, however, is offset by the high viscosity of PEG 20M solutions; whereas the relatively low cost of PEG does not militate against its use on a large scale. Further, increasing PEG molecular weight is likely to increase the partition coefficient of proteins (Albertsson, 1986), which may or may not be advantageous. The increasing symmetry of the binodal as PEG molecular weight increases reflects the anticipated trend when the molecular weights

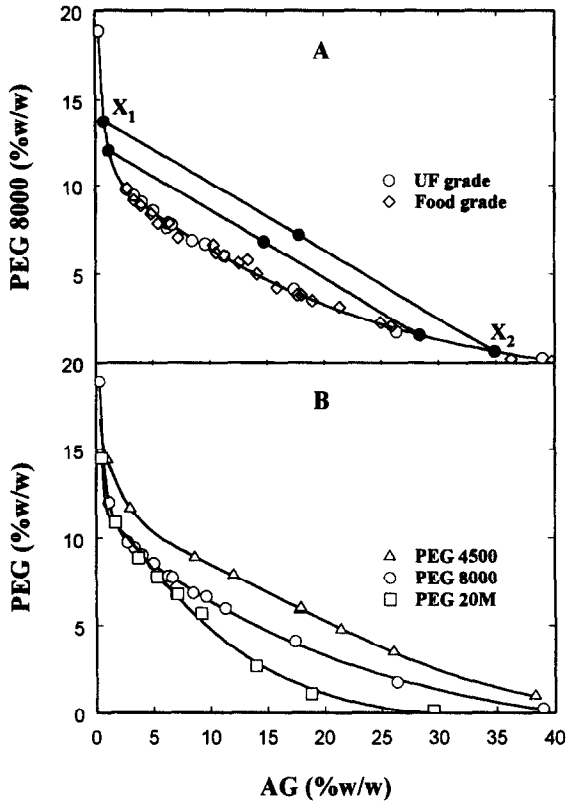

Fig. 1. Phase diagrams. (*A*) Comparison of AG-UF and AG-FG. (*B*) Effect of PEG molecular weight on bindol.

of two-phase-forming polymers approach each other (Albertsson, 1986).

Upper and lower phase densities of AG aqueous two-phase systems with PEG 8000 are similar to those of a typical dextran T500 system (Table 1). The lower phase viscosity of the dextran system is considerably higher than either of the AG lower phases.

Suitability of AG-based systems

The polysaccharides previously used with PEG to produce aqueous two-phase systems are hydroxypropyl starch (Ling *et al.*, 1989; Sturesson *et al.*, 1990), maltodextrins (Szlag and Giuliano, 1988), and more commonly dextran (Kopperschläger and Johansson, 1982; Johansson *et al.*, 1983; Birkenmeier *et al.*, 1986; Tjerneld and Johansson, 1990). Dextran is used on a laboratory scale as very pure, narrow molecular weight range fractions because the ease and completeness of separation of the two phases is inversely related to the polydispersity of the polymers (Albertsson, 1986). However the prohibitive cost of these fractions prevents their use on an industrial scale (Kroner *et al.*, 1984). Crude dextran has been used for large-scale separations (Tjerneld *et al.*, 1985; Larsson and Mattiasson, 1988; Tjerneld and Johansson, 1990). The high viscosity and large polydispersity of crude dextran lead to slow and imperfect phase separations, although these problems might be overcome in technological applications. The starch-based

Table 1. Density, viscosity, and volume ratio of PEG 8000 systems at 25°C

System	Density (g ml^{-1})		Viscosity (cps)		Vol. ratio
	Upper	Lower	Upper	Lower	
16%AG-FG:6%PEG	1.03	1.13	6.55	17.5	1.7
16%AG-UF:6%PEG	1.03	1.13	6.98	20.2	1.6
7%DextranT500: 5%PEG	1.00	1.05	4.51	70.6	1.4

polysaccharides—and to a lesser extent the dextrans—cannot be used for prolonged periods in the presence of many starch-degrading enzymes because they are susceptible to degradation by the enzymes. Arabinogalactan avoids these problems. Even at high concentration (e.g. 40%, w/w) its solutions are relatively non-viscous, with resultant rapid phase separation, and it was not attacked by any of the starch-degrading enzymes. The polydispersity of both grades of AG is particularly low, which results in a predictable and reproducible transition from one to two phases.

We have calculated the current costs of the phase-forming polysaccharides of typical phase systems (Table 2). These calculations show food grade AG to hold an economic advantage. The maltodextrins (Szlag and Giuliano, 1988) are even less expensive but would not be suitable for long-term exposure to starch-degrading enzymes.

Specific optical rotation of AG

Initial optical rotation measurements on AG in conjunction with the construction of tie-lines revealed a pronounced temperature dependence. The specific optical rotation of AG (H_2O, 6.4%, w/w) changed linearly from 9.12 to 4.10°, a 55% decrease, as temperature increased from 30 to 83°C. The effect shows a complete lack of hysteresis on successive heating and cooling (Fig. 2). The specific rotation of a similar dextran T500 solution (H_2O, 5.0%, w/w) showed a similar numerical change from 200.7° at 30°C to 194.0° at 80°C, but this represents a decrease of only about 3%.

The optical rotation of AG is also strongly dependent on the amount of PEG present in solution, as shown in Fig. 3. At 30°C, increasing the PEG 8000 concentration from 0 to

Table 2. Cost of phase-forming polysaccharides in typical aqueous two-phase systems[a]

Polysaccharide	wt.%	$US
Dextran T500	7	35[b]
Crude dextran	7	5.7[b]
Hydroxypropyl starch	14	8.4[c]
Food grade AG	16	1.6[d]
AG-UF	16	12[d]

[a]Based on 1 kg total system containing 5% PEG 8000
[b]Sigma Chemical Co., January 1997
[c]Shearwater Polymers Inc., Huntsville, AL, January 1997
[d]Larex Inc., St. Paul, MN, March 1997

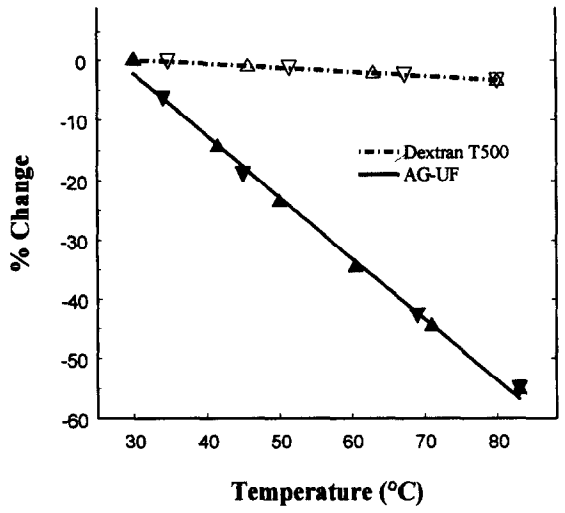

Fig. 2. Relative temperature dependance of AG-UF (H_2O, 6.4%, w/w) and dextran T500 (H_2O, 5.0%, w/w) specific optical rotation. Increasing temperature (AG (▲), dextran (△)), decreasing temperature (AG (▼), dextran (▽)).

8.3% (w/w) caused a decrease of ~30% in specific optical rotation of AG, although the actual change in concentration of AG was only 0.5% (w/w). A repeat of this experiment using dextran T500 (5.0% in water) in place of AG showed no significant change in specific rotation.

The cause of these two effects on the optical rotation of AG solutions is unknown. It probably involves some influence on the conformation of the AG molecule in solution, and may involve an order–disorder transition. Similar behavior is observed in solutions of xanthan (Dea, 1993) and has been attributed to such a transition. As a consequence, tie line determinations for AG systems based on optical rotation measurements are much more difficult than with systems employing, e.g. dextran, which has a much greater specific optical rotation that is relatively insensitive to temperature and to PEG concentration.

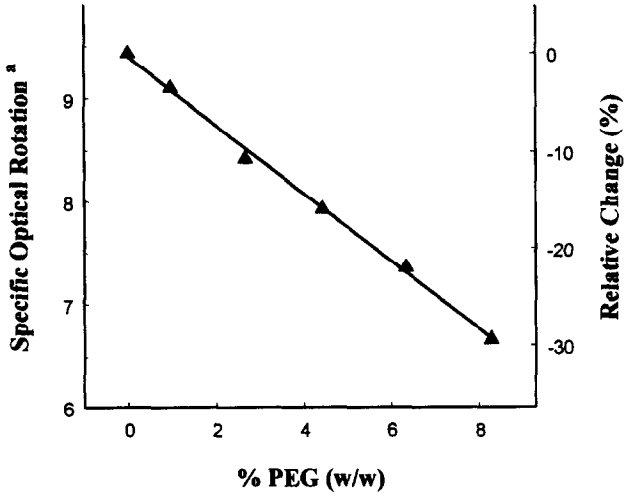

Fig. 3. Change in specific optical rotation of AG in water at 30°C with increasing PEG 8000 concentration. [(a)] Based on known AG concentration (w/w).

Table 3. Effect of pH and NaCl on partition of BSA

pH	Buffer	K_{BSA}
5.0	10 mM Na acetate	0.25
6.0	10 mM Na phosphate	0.30
7.0	10 mM Na phosphate	0.36
8.0	10 mM Na phosphate	0.39
7.0	10 mM Na phosphate + 100 mM NaCl	0.31
7.2	10 mM Tris–HCl	0.37
8.2	10 mM Tris–HCl	0.36

16%AG-UF:6%PEG 8000. Volume ratio = 1.4 (V_{Upper}/V_{Lower}). Approx. 50 mg BSA/10 g system. Temperature 25°C. Assay of both phases by Biuret.

Partitioning of proteins and enzymes

Partitioning of BSA was relatively unaffected by various buffer types, pH values, and salt concentrations (Table 3), varying by about 40%. In contrast, K_{BSA} using either dextran T500 (Sturesson *et al.*, 1990) or Aquaphase PPT (Sturesson *et al.*, 1990) in conjunction with PEG 8000 varies by an order of magnitude over the same range of conditions.

The influences upon K_{BSA} of PEG molecular weight and polymer concentration are shown in Table 4. Partition coefficients for proteins are expected to decrease as total polymer concentration increases (Johansson, 1974; Albertsson, 1986; Sturesson *et al.*, 1990). However, only small changes are observed in the PEG–AG system and no clear trend is evident. Altering the molecular weight of the PEG also has no very significant effect upon the partition coefficient.

Conversion of corn starch to cyclodextrins

In 16% AG:6% PEG 8000 at 25°C, sodium phosphate buffer, pH 6.0, the partition coefficients of α-, β- and γ-cyclodextrins are, respectively, 2.14, 2.14 and 1.96. In this system at 60°C there was significant initial increase in the rate of formation of cyclodextrins compared with single-phase systems. At 3 h the two-phase system showed 40% conversion and after 8 h 45.1% conversion of 5% corn starch to cyclodextrins (16.9% α-, 21.6% β-, 6.6% γ-CD).

Table 4. Influence of PEG molecular weight and polymer concentration on partition of BSA

PEG MW	AG[a]	PEG[a]	Vol. ratio[b]	K^c_{BSA}
8000	6	9	11.3	0.11
	9	11	4.0	0.06
	12	12	3.0	0.06
	16	6	1.4	0.25
20000	6	9	6.7	0.21
	9	11	3.6	0.10
	12	12	2.6	0.09
	16	6	1.0	0.21

Buffer, 10 mM Na acetate; pH 5.0; temp. 25°C
[a]% w/w in total system ±0.1%
[b]V_{Upper}/V_{Lower}
[c]Approx. 50 mg BSA/10 g system

In the absence of polymers the single-phase system produced 30 and 38.5% total CDs (18.1% α-, 15.4% β-, 5.0% γ-CD) at 3 and 8 h, respectively, a 15% reduction in final yield accompanied by a shift in relative abundance of α- and β-CD. The procedure used CGTase in place of α-amylase to liquefy the starch, thus eliminating the need for heat-induced denaturation of α-amylase before beginning CD production. The use of a thermostable CGTase isolated from, e.g. *Thermoanaerobacter* sp. (Starnes, 1990), might allow the conversion to proceed at a higher temperature with subsequent higher yields.

The equilibrium between the three reactions catalyzed by CGTase, viz. formation of CDs, hydrolysis of CDs accompanied by coupling, and disproportionation of oligosaccharides, is commonly manipulated favorably by adding a complexant to precipitate the target CD as it is formed (Armbruster, 1988). In lieu of using organic complexants, and to overcome the difficulty of excluding non-converted starch during crystallization, fractionation by ultra-filtration and adsorption onto synthetic resins have been used to recover CDs directly from the reaction mixture (Armbruster, 1988). The current study indicates some potential for the development of an AG-based aqueous two-phase system for continuous production of cyclodextrins, since CDs partition favorably to the upper phase while non-converted starch remains in the lower phase.

Conversion of corn starch to glucose

Batch conversion of starch to glucose in 0.1 M sodium acetate buffer (pH 5.0) with and without AG and PEG 8000 were compared. Both systems attained greater than 90% conversion after 5 h, though the two-phase system was considerably faster in the early stages, showing 80% conversion after 2 h compared with 65% for the system without polymers. The partition coefficient for glucose in the two-phase system was 1.24. Previous studies by Larsson and Mattiasson (1988) and Hayashida *et al.* (1990) provide convincing testimony to the applicability of aqueous two-phase systems to continuous production of glucose from starch. Arabinogalactan may provide a more economical alternative to the fractionated dextrans in such systems, owing to its narrow molecular weight range, low viscosity at high concentrations, and relatively low cost.

There is current industrial interest in aqueous protein–polysaccharide systems, and these provide another possible industrial rôle for an inexpensive source of pure arabinogalactan of narrow molecular weight range. The concentration of skimmed milk proteins using polysaccharides, such as arabinogalactan and pectin, has been achieved by membraneless isobaric osmosis (Antonov *et al.*, 1982). In this process arabinogalactan demonstrated superior ability to concentrate protein but at the expense of a greatly increased concentration requirement for the polysaccharide relative to esterified pectin. Phase separation of proteins and neutral polysaccharides is sensitive to salt concentrations; in these systems phase separation usually occurs if the salt

concentration is higher than 0.1 M (Tolstoguzov, 1988). A theoretical treatment of protein–polysaccharide systems (Clewlow *et al.*, 1995) indicates that the two important properties of polymers required for predicting behavior in incompatible mixtures are number average molecular weight and the degree of polydispersity. A narrow distribution of molecular weights permits more accurate prediction of behavior.

ACKNOWLEDGEMENTS

This work was supported by Larex, Inc., St. Paul, MN. The authors would like to thank Brendon Gill, University of Waikato, Hamilton, New Zealand, for experimental assistance.

REFERENCES

Albertsson, P.-Å. (1986). *Partition of Cell Particles and Macromolecules*, 3rd ed. New York: John Wiley & Sons.

Antonov, Y.A., Grinberg, V.Y., Zhuravskaya, N.A. & Tolstoguzov, V.B. (1982) Concentration of the proteins of skimmed milk by membraneless isobaric osmosis *Carbohydr. Polym.*, **2**, 81–90.

Armbruster, F. C. (1988). *Proceedings of the Fourth International Symposium on Cyclodextrins*, pp. 33–39, Kluwer Academic Publishers.

Birkenmeier, G., Kopperschläger, G. & Johansson, G. (1986) Separation and studies of serum proteins with aid of aqueous two-phase systems containing dyes as affinity ligands. *Biomed. Chromatogr.*, **1**(2), 64–77.

Clewlow, A. C., Rowe, A. J., & Tombs, M. P. (1995). Pectin-gelatin phase separation: the influence of polydispersity. In S. E. Harding, S. E. Hill, & J. R. Mitchell (Eds.), *Biopolymer Mixtures* (pp. 173–191). Nottingham University Press, Nottingham.

Dea, I. C. M. (1993). Conformational origins of polysaccharide solution and gel properties. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial Gums: Polysaccharides and Their Derivatives* (pp. 21–52). Academic Press, Inc., San Diego.

Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T. & Schaffer, R. (1981) A candidate reference method for determination of total protein in serum. I. Development and validation *Clin. Chem.*, **27**(10), 1642–1650.

Hayashida, K., Kunimoto, K., Shiraishi, F., Kawakami, K. & Arai, Y. (1990). Enzymatic hydrolysis of soluble starch in a polyethylene glycol-dextran aqueous two-phase system. *J. Ferment. Bioeng.*, **69**(4), 240–243.

Johansson, G. (1974) Partition of proteins and micro-organisms in aqueous biphasic systems. *Mol. Cell. Biochem.*, **4**(3), 169–180.

Johansson, G., Kopperschläger, G. & Albertsson, P.-Å. (1983) Affinity partitioning of phosphofructokinase from Baker's yeast using polymer-bound cibacron blue F3G-A *Eur. J. Biochem.*, **131**, 589–594.

Kopperschläger, G. & Johansson, G. (1982) Affinity partitioning with polymer-bound cibacron blue F3G-A for rapid, large-scale purification of phosphofructokinase from Baker's yeast *Anal. Biochem.*, **124**, 117–124.

Kroner, K.H., Hustedt, H., Kula, M.R. (1982) Evaluation of crude dextran as phase-forming polymer for the extraction of enzymes in aqueous two-phase systems in large scale *Biotechnol. Bioeng.*, **24**, 1015–1045.

Kroner, K.H., Hustedt, H., Kula, M.R. (1984) Extractive enzyme recovery: economic considerations *Process Biochem.*, **24**, 170–179.

Larsson, M., Arasaratnam, V., Mattiasson, B. (1988) Integration of bioconversion and downstream processing: starch hydrolysis in an aqueous two-phase system *Biotechnol. Bioeng.*, **33**, 758–766.

Larsson, M., Mattiasson, B. (1988) Characterization of aqueous two-phase systems based on polydisperse phase forming polymers: enzymatic hydrolysis of starch in a PEG-starch aqueous two-phase system *Biotechnol. Bioeng.*, **31**, 979–983.

Ling, T.G.I., Nilsson, H., Mattiasson, B. (1989) Reppal PES—A starch derivative for aqueous two-phase systems *Carbohydr. Polym.*, **11**, 43–54.

Owens, H.S. (1940) The viscosities of arabinogalactan solutions *J. Am. Chem. Soc.*, **62**, 930–932.

Prescott, J.H., Enriques, P., Jung, C., Menz, E., Groman, E.V. (1995) Larch arabinogalactan for hepatic drug delivery: Isolation and characterization of a 9 kDa arabinogalactan fragment *Carbohydr. Res.*, **278**(113), 113–128.

Starnes, R.L. (1990) Industrial potential of cyclodextrin glycosyl transferases *Cereal Foods World*, **35**(11), 1094–1097.

Sturesson, S., Tjerneld, F., Johansson, G. (1990) Partition of macromolecules and cell particles in aqueous two-phase systems based on hydroxypropyl starch and poly(ethylene glycol) *Appl. Biochem. Biotechnol.*, **26**(3), 281–295.

Szlag, D.C., Giuliano, K.A. (1988) A low-cost aqueous two phase system for enzyme extraction *Biotechnol. Tech.*, **2**(4), 277–282.

Tilden, E.B. & Hudson, C.S. (1942). Preparation and properties of the amylases produced by *Bacillus macerans* and *Bacillus polymyxa*, *J. Bacteriol.*, **43**, 527–544.

Tjerneld, F., Johansson, G. (1990) Aqueous two-phase systems for biotechnical use *Bioseparation*, **1**, 255–263.

Tjerneld, F., Persson, I., Albertsson, P.-Å. (1985) Enzymatic hydrolysis of cellulose in aqueous two-phase systems. I. Partition of cellulases from *Trichoderma* reesei *Biotechnol. Bioeng.*, **27**, 1036–1043.

Tolstoguzov, V.B. (1988) Concentration and purification of proteins by means of two-phase systems: Membraneless osmotic process *Food Hydrocol.*, **2**(3), 195–207.

Vernau, J., Kula, M.R. (1990) Extraction of proteins from biological raw material using aqueous polyethylene glycol-citrate phase systems *Biotechnol. Appl. Biochem.*, **12**(4), 397–404.

Whistler, R. L. (1993). Hemicelluloses. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial Gums: Polysaccharides and Their Derivatives*, 3rd ed. (pp. 295–308). Academic Press, Inc., San Diego.