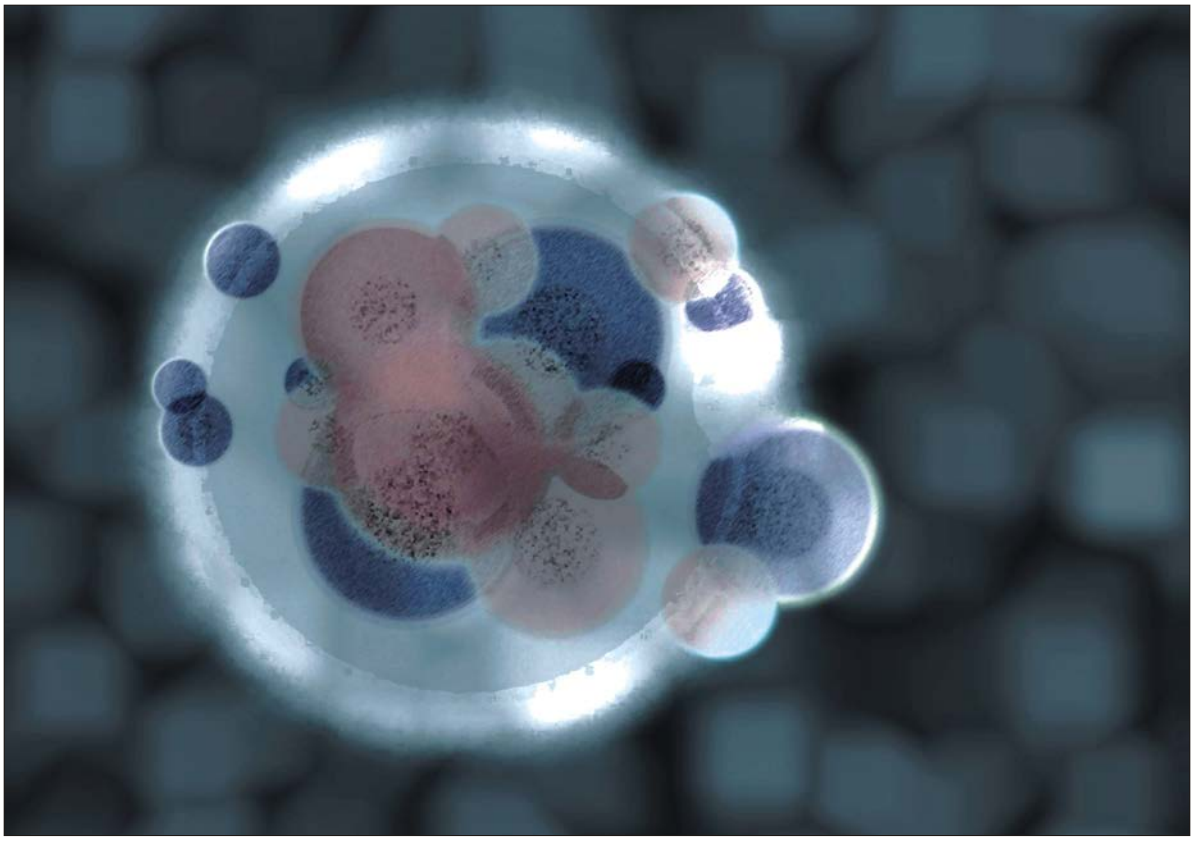

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 8 (2): 288-297, 2008
ISSN 1727-3048
© 2008 Asian Network for Scientific Information

Enzyme Partitioning Using PEG-*Anacardium occidentale* L. Exudate Gum Polysaccharide Aqueous Two-Phase Systems

[1]E.B. Tambourgi, [1]L.A. Sarubbo, [1,2]L.A. Oliveira, [2]J.L. Lima-Filho, [2,3]A.L.F. Porto,
[4]G.M. Campos-Takaki, [5]B. Liu, [5]C.J. Knill and [5,6]J.F. Kennedy
[1]Departamento de Engenharia de Sistemas Químicos (DESQ),
Faculdade de Engenharia Química (FEQ), Cidade Universitária,
Universidade Estadual de Campinas (UNICAMP), Barão Geraldo,
CEP 13083-852, Campinas, São Paulo (SP), Brazil
[2]Laboratório de Imunopatologia, Keizo Asami (LIKA),
Universidade Federal de Pernambuco (UFPE), Cidade Universitária, CEP 50670-901,
Recife, Pernambuco (PE), Brazil
[3]Departamento de Morfologia e Fisiologia Animal,
Universidade Federal Rural de Pernambuco (UFRPE),
Dois Irmãos, CEP 52171-900, Recife, Pernambuco (PE), Brazil
[4]Departamento de Química, Núcleo de Pesquisas em Ciências Ambientais (NPCIAMB),
Universidade Católica de Pernambuco (UNICAP), Ruo do Principe, Boa Vista,
CEP 50050-590, Recife, Pernambuco (PE), Brazil
[5]Chembiotech Laboratories, Institute of Research and Development,
University of Birmingham Research Park, 97 Vincent Drive, Edgbaston,
Birmingham, West Midlands, B15 2SQ, UK
[6]Birmingham Carbohydrate and Protein Technology Group, School of Chemistry,
University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK

Abstract: Effects of PEG molecular weight, system polymer concentrations (tie-line lengths), pH and sodium chloride concentration, on the partition coefficient of trypsin in PEG-purified *Anacardium occidentale* L. (cashew tree) exudate gum polysaccharide aqueous two-phase systems have been investigated. Changes in PEG molecular weight, tie-line length and pH, had relatively little effect on trypsin partitioning, with partition coefficients (K) < 0.3, i.e., trypsin partitioned preferentially into the *A. occidentale* L. gum (lower) phase. However, addition of sodium chloride (0.1 M) resulted in dramatic increases in K values with increasing pH using the PEG 4000 (9% w/w)-purified *A. occidentale* L. gum (18% w/w) system, increasing to 3.70 at pH 7.0 and 9.77 at pH 8.0, i.e., trypsin partitioned preferentially into the PEG 4000 (upper) phase. Relative trypsin activities in the phases were investigated for these systems (with different sodium chloride levels) and high activities were obtained at pH 7.0 (70.9%) and pH 8.0 (90.9%) in 0.1 M sodium chloride, which in conjunction with their K values, clearly demonstrates their suitability as lower cost enzyme purification procedures (compared with PEG-dextran).

Key words: *Anacardium occidentale* L., partition coefficient, partitioning, PEG, trypsin

INTRODUCTION

Aqueous liquid-liquid two-phase extraction has been utilised for many years as a separation/ purification methodology with wide application in areas of biochemistry, cell biology and biotechnology (Walter *et al*., 1985; Albertsson, 1986; Walter and Johansson, 1986; Zaslavsky, 1995). Compared with other commonly used techniques, partitioning in aqueous two-phase systems has several advantages, such as easy scale-up and the ability to handle particulate materials and process streams continuously (Carlson, 1988).

Corresponding Author: J.F. Kennedy, Birmingham Carbohydrate and Protein Technology Group, School of Chemistry, University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK
Tel: +44 (0)121 414 7029

Aqueous two-phase systems are composed of two substances dissolved in water above a certain critical phase separating concentration and the first kind of such systems, used for protein partitioning, were composed of water, a water-soluble organic solvent and a salt. The second kind of aqueous two-phase systems consist of a polymeric substance and a salt in water. The most commonly used systems are based on poly (ethylene glycol) (PEG) and potassium phosphate or ammonium sulphate and for economical reasons these systems have been used for large-scale processes, particularly enzyme purification (Kula *et al*., 1989; Veide *et al*., 1983; Kroner *et al*., 1982, 1984; Tjerneld *et al*., 1987a, b; Tjerneld and Johansson, 1990; Cascone *et al*., 1991; Schmidt *et al*., 1994; Queiroz *et al*., 1995; Sinha *et al*., 1996). However, their disadvantages are that they can damage fragile proteins and also that they result in waste disposal problems (Vernau and Kula, 1990). The third kind of aqueous two-phase systems are composed of two polymers in water. The most commonly used are PEG-dextran systems, which offer good flexibility in terms of water content and salt concentration and the localisation of the respective polymers in different phases offers the possibility to restrict chemical groups on the partitioned substrate to one phase by direct interaction with the corresponding polymer (Tjerneld and Johansson, 1990).

Partitioning of bioactive materials in aqueous two-phase systems is affected by the surface properties of bioactive materials such as surface net charge, molecular weight, shape, surface hydrophobicity and the existence of specific binding sites and the partition coefficient is affected by the concentration and phase-forming polymer properties, incorporated ions, pH and the affinity of the macromolecule for the phase-forming polymer (Albertsson, 1986). The electrical potential between the upper and the lower phases of aqueous two-phase systems are created by the non-homogeneous distribution of ions in the upper and lower phases (Han and Lee, 1997).

By using polymer-polymer aqueous two-phase systems it is possible to have phases with high water contents (80-90% w/v) and low interfacial tension, with good solubility for proteins and other biomacromolecules. PEG-dextran systems have been utilised for the separation of many target substances on a laboratory scale (Albertsson *et al*., 1990; Park and Wang, 1991; Schmidt *et al*., 1994; Han and Lee, 1997; Gündüz and Korkmaz, 2000; Lin *et al*., 2003). However, larger scale biotechnological applications have been slow to develop due to the inherently high costs of fractionated dextrans of high purity and well defined molecular weight profiles (with relatively low degrees of polydispersity), which are necessary for good reproducibility (Tjerneld and Johansson, 1990). Cheaper crude dextrans have been investigated, however they contain high molecular weight fractions that result in high viscosity and therefore associated handling difficulties (Kroner *et al*., 1982; Tjerneld *et al*., 1985). Other sources of purified dextran (Ghosh *et al*., 2004) and dextran derivatives (including hydroxypropyl-, carboxymethyl-, diethylaminoethyl- and sulphated dextran) have also been used for two-phase systems (Albertsson, 1986).

Investigations have been performed into the formation and application of aqueous two-phase systems using less expensive carbohydrate polymers, such as pullulan (Nguyen *et al*., 1988), cellulose derivatives (Albertsson, 1986; Tjerneld, 1989; Skuse *et al*., 1992), starch derivatives (Tjerneld *et al*., 1986, 1987b; Sturesson *et al*., 1990; Venâncio *et al*., 1993; Almeida *et al*., 1998; Wu *et al*., 2001), maltodextrin (starch hydrolysate) (Szlag and Guiliano, 1988; Atkinson and Johns, 1994; da Silva and Meirelles, 2000a, b), agarose (Medin and Jansson, 1993), guar gum (Venâncio *et al*., 1995), locust bean gum (Venâncio *et al*., 1996) and larch arabinogalactan (Christian *et al*., 1998). Information on the structural characteristics of these different carbohydrate-based polymeric biomaterials and their derivatives can be obtained from many sources (Kennedy, 1974; Kennedy and White, 1983; Kennedy, 1988; de Belder, 1993; Kennedy *et al*., 1995; Carioca *et al*., 1996; Heinze and Glasser, 1998; Barsby *et al*., 2001; Yuryev *et al*., 2002; Gotlieb and Capelle, 2005; Knill and Kennedy, 2005; Collins, 2006).

The potential utilisation of the exudate gum from *Anacardium occidentale* L. (cashew) as an aqueous-phase-forming polymer and its capacity for protein (bovine serum albumin) separation/partitioning in aqueous two-phase systems with PEG (effect of PEG molecular weight, pH, temperature and relative polymer concentrations (tie-line length)), has been assessed previously (Sarubbo *et al*., 2000; Oliveira *et al*., 2001). *A. occidentale* L. is found in many tropical and subtropical countries, especially Brazil, Venezuela, India, Malaysia and Papua New Guinea. Its exudate gum, produced in epithelial cells which border the gum ducts (Nair *et al*., 1983), is part of the plants biochemical defences (Marques and Xavier-Filho, 1991) and has found local use as a substitute for gum Arabic (de Paula and Rodrigues, 1995), since it has some similar physicochemical characteristics (Mothé and Rao, 1999, 2000).

The primary polysaccharide component of *A. occidentale* L. exudate gum is a highly branched acidic hetero-galactan (Fig. 1) (a complex mixture of Ca, Mg, K

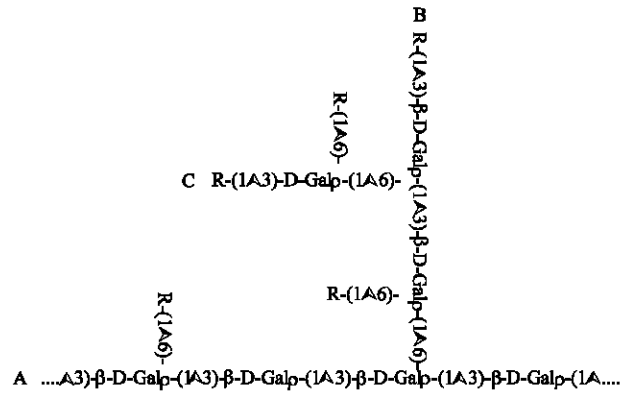

Fig. 1: Structural representation of a fragment of purified *A. occidentale* L. exudate gum polysaccharide (Anderson and Bell, 1975; Sarubbo *et al*., 2000) {A = primary backbone; B and C = side-chains; R = D-Gal, D-Glc, L-Ara, L-Rha, D-Man, D-GlcA or 4-O-Me-D-GlcA non-reducing end groups}

and Na salts, $M_W \sim 1.0\text{-}1.5 \times 10^4$), which has a (1➞3)-linked β-D-Gal*p* backbone (A chain in Fig. 1), with (1➞6)-linked …➞3)-β-D-Gal*p*-(1➞… galactan side chains (B and C chains in Fig. 1) and D-Gal, D-Glc, L-Ara, L-Rha, D-Man, D-GlcA and 4-O-Me-D-GlcA non-reducing end groups and D-Xyl residues have also been detected in some samples (Bose and Biswas, 1970; Anderson *et al*., 1974; Anderson and Bell, 1975; de Paula and Rodrigues, 1995; de Pinto *et al*., 1995; Zakaria and Rahman, 1996; de Paula *et al*., 1998; Menestrina *et al*., 1998; Mothé and Rao, 1999; Silva *et al*., 2004). Brazilian *A. occidentale* L. exudate gum (utilised in this study) is composed (w/w) of Gal (~ 72-73%), Glc (~ 11-14%), Ara (~ 4-5%), Rha (~3-4 %), Man (~ 0-1%), GlcA/4-O-Me-GlcA (~ 5-6%) (Zakaria and Rahman, 1996; de Paula *et al*., 1998; Menestrina *et al*., 1998; Mothé and Rao, 1999; Silva *et al*., 2006).

The effects of PEG molecular weight, relative polymer concentrations (tie-line length), pH and sodium chloride concentration, on the partition coefficient and enzyme activity of trypsin in PEG-*Anacardium occidentale* L. exudate gum polysaccharide aqueous two-phase systems are presented in this reserch.

MATERIALS AND METHODS

***A. occidentale* L. exudate gum polysaccharide isolation:** Crude natural exudate gum (~ 1 kg) was collected from cultivated *A. occidentale* L. trees (common-type yellow cashew producers, ~ 20 years old) in various locations within the sate of Pernambuco (PE), Brazil. Clear exudate gum nodules (~ 1-10 g), free of bark, were selected for purification as Na salts using a method based on those of Villain-Simonnet *et al*. (1999 and 2000), as detailed below. Crude gum was dissolved in sodium chloride solution (NaCl, 0.5 M, 25±1°C) to give a gum concentration of ~5 g L^{-1}. The resultant crude gum solution was centrifuged (25 min, 30000 G, 25±1°C) and the supernatant filtered (0.45 μm). The polysaccharide was precipitated from the filtered supernatant using ethanol (giving a final ethanol concentration of 55% v/v), isolated by suction filtration, washed with ethanol: sodium chloride solution of increasing ethanol concentration (75-100% v/v ethanol: 25-0% v/v 0.5 M NaCl) and dried under vacuum for 48 h at 30°C. Purified *A. occidentale* L. exudate gum polysaccharide recovery was ~ 90% w/w (which is in close agreement with Anderson *et al*. (1974) and Marques and Xavier-Filho (1991).

Determination of phase diagrams: Phase diagrams were determined according to the method of Albertsson (1986), as detailed in previous investigations (Sarubbo *et al*., 2000; Oliveira *et al*., 2001) (Fig. 2). The binodial line, the demarcation between PEG (4000 and 8000)-purified *A. occidentale* L. exudate gum polysaccharide compositions showing monophasic and biphasic behaviour, was obtained by direct observation of two-phase formation for triplicate solutions of varying polymer concentrations (Table 1) in phosphate buffer (15 mM, pH 6.0), which were mechanically stirred for several minutes to ensure equilibrium conditions and the phases generated were allowed to separate over a 24 hour period in beakers placed in a constant temperature bath (27±2°C). PEG (4000 and 8000)-purified *A. occidentale* L. exudate gum polysaccharide two-phase systems (Table 1) were prepared from stock solutions of PEG 4000 and 8000 (50% w/w) and purified *A. occidentale* L. exudate gum polysaccharide (30% w/w) in 15 mM phosphate buffer (pH 6.0). Systems that displayed a distinct phase-phase

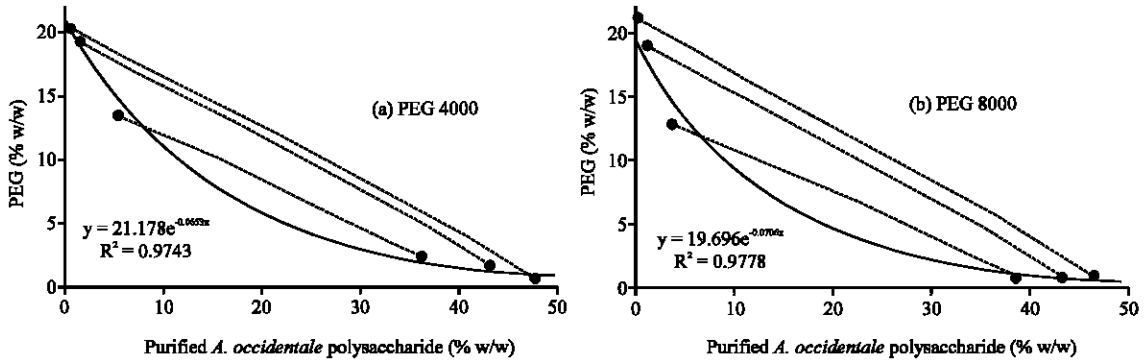

Fig. 2: Phase diagrams for PEG-purified *A. occidentale* L. exudate gum polysaccharide systems (27±2°C, pH 6.0): (a) PEG 4000; (b) PEG 8000 (Sarubbo *et al*., 2000; Oliveira *et al*., 2001)

Table 1: Initial system and final phase compositions (% w/w) of PEG-purified *A. occidentale* L. exudate gum polysaccharide systems (27±2°C, pH 6.0): (a) PEG 4000; (b) PEG 8000; (Sarubbo *et al*., 2000; Oliveira *et al*., 2001)

(a) PEG 4000

System composition			Upper phase		Lower phase	
A. occidentale (% w/w)	PEG (% w/w)	V_t	*A. occidentale* (% w/w) *	PEG (% w/w) *	*A. occidentale* (% w/w) *	PEG (% w/w) *
18	9	1.7	5.5	13.5	36.4	2.4
20	11	1.6	1.7	19.2	43.2	1.7
22	13	1.5	0.6	20.2	47.8	0.6

(b) PEG 8000

System composition			Upper phase		Lower phase	
A. occidentale (% w/w)	PEG (% w/w)	V_t#	*A. occidentale* (% w/w) *	PEG (% w/w) *	*A. occidentale* (% w/w) *	PEG (% w/w) *
16	9	2.2	3.6	13.3	39.2	0.9
18	11	1.9	1.3	19.4	43.9	0.9
20	13	1.8	0.4	21.6	47.4	1.0

* All quoted values (%w/w) are derived from the mean of triplicate analyses (all with%variation values<5%) (% variation = 100 x (standard deviation/mean), # V_t = system volume ratio = volume of upper phase/volume of lower phase

interface were considered biphasic. Polymer concentrations in the upper and lower phases were determined (Table 1): PEG concentration using the method of Skoog (1979) and *A. occidentale* L. exudate gum polysaccharide concentration using the 3,5-dinitrosalicylic acid assay for reducing sugars (Miller, 1959; White and Kennedy, 1981; Chaplin, 1994), using purified *A. occidentale* L. exudate gum polysaccharide and D-galactose as standards. PEG 4000 and PEG 8000 were obtained from Sigma-Aldrich Company Ltd., Poole, Dorset, UK.

Trypsin partitioning: PEG (4000 and 8000)-purified *A. occidentale* L. exudate gum polysaccharide two-phase systems (Table 1) were prepared from stock solutions of PEG 4000 and 8000 (50% w/w) and purified *A. occidentale* L. exudate gum polysaccharide (30% w/w) in 15 mM phosphate buffer (pH 6.0, 7.0 and 8.0). Sodium chloride (NaCl) was directly dissolved into replicate systems to afford salt concentrations of 0.1 and 1.0 M as required. The total weights of the final systems used were 4 g. Enzyme partitioning experiments were performed in triplicate at 27±2°C (constant temperature bath) by mixing the systems with trypsin solution (2 mg mL^{-1}, 400 μL). The systems were vortex mixed (5 min) and centrifuged (5 min, 236 G, 25±1°C) to obtain two clear phases. Thus, triplicate system variables included 3 different tie-line lengths (relative polymer concentrations/compositions), 2 PEG molecular weights, 3 pH's and 3 salt concentrations. Trypsin (from bovine pancreas, EC 3.4.21.4) was obtained from Sigma-Aldrich Company Ltd., Poole, Dorset, UK and had an activity of > 10,000 BAEE U/mg protein. [Unit definition: 1 U corresponds to the amount of enzyme which increases the absorbance at 253 nm (ΔA_{253}) by 0.001 per minute at pH 7.6 and 25°C using N-benzoyl-L-arginine ethyl ester (BAEE) as substrate].

Determination of trypsin partition coefficients (K): Protein (trypsin) concentrations were determined (in triplicate) in the upper phases according to the method of

Bradford (1976) and in the lower phases calculated by mass balance, due to high viscosity, according to the methods of Venâncio *et al.* (1993) and Almeida *et al.* (1998). Partition coefficient (K) was defined as the ratio between trypsin concentration in the upper (PEG) and lower (*A. occidentale*) phases. Effects of system composition (PEG molecular weight, tie-line length, pH and sodium chloride) on trypsin partition coefficients are presented Table 2 and data of specific interest presented graphically in Fig. 3.

Determination of trypsin activity: Trypsin activity in the upper and lower phases of selected systems (chosen based upon their partition coefficients, determined using the methodology detailed in the previous section) was measured in triplicate according to the method of Ginther (1979) using azocasein (Sigma-Aldrich Company Ltd., Poole, Dorset, UK, 1.0% w/w in 0.2 M Tris-HCl buffer, pH 7.2, containing 1.0 mM $CaCl_2$). [Unit definition: 1 U corresponds to the amount of enzyme which increases the absorbance at 440 nm (ΔA_{440}) by 1.0 per hour at pH 7.2 and 25°C using azocasein as substrate]. Determined trypsin activities for the analysed systems are presented in Table 3 (quoted values are relative trypsin activity (%), with 100% activity being that measured for trypsin in 15 mM sodium phosphate buffer (pH 6.0, 7.0 or 8.0), i.e., the appropriate polymer-free control solutions).

RESULTS AND DISCUSSION

Effect of system composition on trypsin partition coefficient (K): Effects of system composition (PEG molecular weight, tie-line length, pH and sodium chloride) on trypsin partition coefficients are presented in Table 2. In the absence of sodium chloride, the investigated systems were not significantly influenced by PEG molecular weight, pH or tie-line length (all determined K-values < 0.3, i.e., trypsin partitioned preferentially into the *A. occidentale* L. gum (lower) phase). Partition behaviour is more sensitive to changes in polymer molecular weight for proteins > 50 kDa. The relatively low molecular weight of trypsin (25 kDa) may account for this, the same behaviour being observed for cutinase (22 kDa) partitioning in PEG-starch systems (Almeida *et al.*, 1998). Increases in polymer concentrations (tie-line length) either resulted in a reduction in K values (or had relatively little effect). Increasing PEG concentration causes molecular exclusion of trypsin from the upper to the lower phase (Sturesson *et al.*, 1990; Almeida *et al.*, 1998).

Addition of sodium chloride can be utilised to significantly increase protein partition coefficients in aqueous two-phase systems, an effect that has been attributed to possible hydrophobic interactions (Cascone *et al.*, 1991; Schmidt *et al.*, 1994). In the

Table 2: Effect of system composition on trypsin partition coefficients (K) in PEG-purified *A. occidentale* L. exudate gum polysaccharide aqueous two-phase systems (27±2°C): (a) PEG 4000; (b) PEG 8000

(a) PEG 4000

System composition			Partition coefficient (K) *		
A. occidentale (% w/w)	PEG (% w/w)	NaCl (M)	pH 6.0	pH 7.0	pH 8.0
18	9	0	0.11	0.07	0.09
		0.1	0.24	3.70	9.77
		1.0	0.62	0.09	0.45
20	11	0	0.04	0.10	0.07
		0.1	0.23	0.21	1.54
		1.0	0.91	0.88	0.70
22	13	0	0.03	0.09	0.11
		0.1	0.18	0.19	0.92
		1.0	1.12	0.81	0.40

(b) PEG 8000

System composition			Partition coefficient (K) *		
A. occidentale (% w/w)	PEG (% w/w)	NaCl (M)	pH 6.0	pH 7.0	pH 8.0
16	9	0	0.05	0.27	0.06
		0.1	0.09	0.59	2.02
		1.0	0.47	0.87	0.66
18	11	0	0.03	0.16	0.09
		0.1	0.06	0.49	0.63
		1.0	0.34	0.83	0.58
20	13	0	0.03	0.11	0.08
		0.1	0.05	0.40	0.47
		1.0	0.46	0.64	0.50

* All quoted K values are derived from the mean of triplicate analyses (all with % variation values < 10%) [% variation = 100 x (standard deviation/mean)]

Table 3: Effect of sodium chloride on relative trypsin activities (%) for selected PEG-purified *A. occidentale* L. exudate gum polysaccharide systems (25±2°C)

System composition			Relative trypsin activity (%) *					
			pH 6.0		pH 7.0		pH 8.0	
A. occidentale (% w/w)	PEG (% w/w)	NaCl(M)	Upper phase	Lower phase	Upper phase	Lower phase	Upper phase	Lower phase
		0	30.0	21.8	49.0	17.1	80.5	29.8
18	9 (PEG 4000)	0.1	24.7	21.5	49.0	70.9	29.9	90.9
		1.0	17.6	22.4	27.5	81.3	44.0	46.9
		0	2.2	0.5	29.7	22.1	51.9	19.5
16	9 (PEG 8000)	0.1	0.9	1.5	33.4	21.1	39.0	37.5
		1.0	0.7	1.4	36.3	93.1	26.0	69.7

* All quoted values (%) are derived from the mean of triplicate analyses (all with % variation values < 5%) [% variation = 100 x (standard deviation/mean)]

investigated systems, addition of sodium chloride (0.1 M) resulted in dramatic increases in K values with increasing pH using the PEG 4000 (9% w/w)-purified *A. occidentale* L. gum (18% w/w) system, increasing to 3.70 at pH 7.0 and 9.77 at pH 8.0 (Table 2, Fig. 3), i.e., trypsin partitioning preferentially into the PEG 4000 (upper) phase.

Effect of system composition on relative trypsin activity: The upper and lower phases from the first tie-line lengths for each of the PEG 4000 and PEG 8000-purified *A. occidentale* L. gum polysaccharide systems were selected for determination of relative trypsin activity (Table 3), since aspects of these systems demonstrated the greatest effect on partition coefficients (Table 2, Fig. 3). In general, higher relative trypsin activities were obtained in the pH 7.0 and 8.0 systems and higher molecular weight systems (PEG 8000) resulted in lower relative trypsin activities than their lower molecular weight (PEG 4000) counterparts (Table 3). Similar behaviour was observed for lipolytic activity recovery in PEG-phosphate systems (Queiroz *et al*., 1995). The maximum relative trypsin activity was obtained with PEG 8000 at pH 7.0 in 1.0 M NaCl (93.1%), in the lower phase. High relative trypsin activities were obtained for the lower phases of the systems with the highest partition coefficients in Table 2 (i.e., the most enzyme material in the upper phases), namely 70.9% at pH 7.0 and 90.9% at pH 8.0 (in 0.1 M sodium chloride), clearly demonstrating the suitability of these systems for trypsin partitioning/ purification.

Certain systems (Table 3) have combined upper and lower phase relative trypsin activities of > 100%, which can be due to a number of distinct factors that affect enzyme activity by altering access to active sites, such as microviscosity (Lindeström-Lang and Schellman, 1959). Increasing solvent viscosity has been shown to decrease enzyme catalytic rate constants for hyaluronate lyase, lactate dehydrogenase and trypsin (Laurent, 1971; Demchenko *et al*., 1989), however contradictory results, i.e., increases in activity with increasing viscosity, have been observed for selected enzymes, including trypsin,

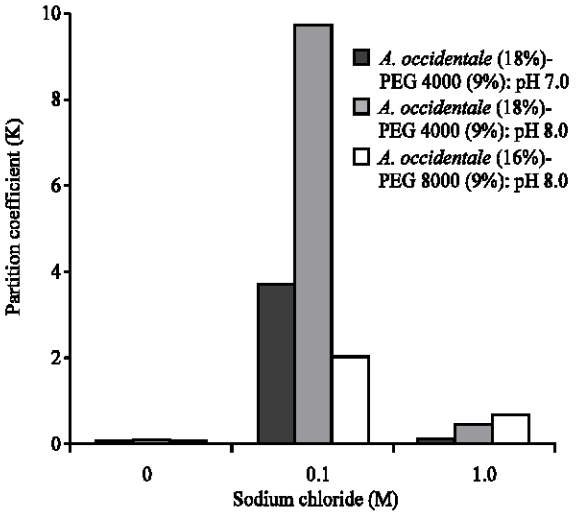

Fig. 3: Effect of sodium chloride addition on trypsin partition coefficient in selected PEG-purified *A. occidentale* L. exudate gum polysaccharide systems (27±2°C)

with polyvinylpyrrolidone (Trón *et al*., 1976; Van der Sanden *et al*., 1995) and PEG 400 and 6000 (Van der Sanden *et al*., 1995; Sinha *et al*., 1996). Charged molecules show a strong viscosity dependence on ionic strength. *A. occidentale* L. gum polysaccharide is composed of ~ 5-6% w/w GlcA/4-O-Me-GlcA residues (Zakaria and Rahman, 1996; de Paula and Rodrigues, 1995; de Paula *et al*., 1998; Menestrina *et al*., 1998; Mothé and Rao, 1999; Silva *et al*., 2006). Ionisation of the GlcA/4-O-Me-GlcA carboxyl groups above pH 7.0 could result in changes in viscosity, as observed for mucilage gum containing similar uronic acid levels (Trachtenberg and Mayer, 1982; Medina-Torres *et al*., 2000).

CONCLUSIONS

Manipulation of the PEG-purified *A. occidentale* L. gum system composition can be utilised for successful trypsin purification/fractionation, since bulk inactive

material has been successfully separated from small quantities of material with high relative trypsin activity. Such systems therefore have great potential as lower cost enzyme purification procedures (compared with PEG-dextran).

ACKNOWLEDGMENTS

The authors would like to express their thanks to FAPESP, CAPES and the British Council, for providing the financial support for these investigations.

REFERENCES

Albertsson, P.Å., 1986. Partition of Cell Particles and Macromolecules. 3rd Edn. Wiley, New York.

Albertsson, P.Å., G. Johansson and F. Tjerneld, 1990. Aqueous Two-Phase Separations. In: Separation Processes in Biotechnology, Asenjo, J.A. (Ed.). Marcel Dekker, New York, pp: 287-318.

Almeida, M.C., A. Venâncio, J.A. Teixeira and M.R. Aires-Barros, 1998. Cutinase purification on poly (ethylene glycol)-hydroxypropyl starch aqueous two-phase systems. J. Chromatogr. B, 711: 151-159.

Anderson, D.M.W., P.C. Bell and J.R.A. Millar, 1974. Composition of gum exudates from *Anacardium occidentale*. Phytochemistry, 13: 2189-2193.

Anderson, D.M.W. and P.C. Bell, 1975. Structural analysis of the gum polysaccharide from *Anacardium occidentale*. Anal. Chim. Acta, 79: 185-197.

Atkinson, L. and M.R. Johns, 1994. Trypsin and α chymotrypsin partitioning in polyethylene glycol/maltodextrin aqueous two-phase systems. Trans. Institution Chem. Eng., 72: 106-112.

Barsby, T.L., A.M. Donald and P.J. Frazier, 2001. Starch: Advances in Structure and Function. Royal Society of Chemistry, Cambridge.

Bose, S. and S.Y. Biswas, 1970. *Anacardium occidentale* gum I: Immunochemical and hydrolysis studies. Indian J. Biochem., 7: 68-72.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Carioca, J.O.B., H.L. Arora, P.V. Pannir Selvam, F.C.A. Tavares, J.F. Kennedy and C.J. Knill, 1996. Industrial utilisation of starch and its derived products in Brazil. Starch/Stärke, 48: 322-326.

Carlson, A., 1988. Factors influencing the use of aqueous two-phase partition for protein purification. Separation Sci. Technol., 23: 785-817.

Cascone, O., B.A. Andrews and J.A. Asenjo, 1991. Partitioning and purification of thaumatin in aqueous two-phase systems. Enzyme Microbial. Technol., 13: 629-635.

Chaplin, M.F., 1994. Monosaccharides. In: Carbohydrate Analysis: A Practical Approach, Chaplin, M.F. and J.F. Kennedy (Eds.). 2nd Edn. Oxford University Press, Oxford, pp: 1-41.

Christian, T.J., M. Manley-Harris and G.N. Richards, 1998. A preliminary study of the use of larch arabinogalactan in aqueous two-phase systems. Carbohydrate Polymers, 35: 7-12.

Collins, P.M., 2006. Dictionary of Carbohydrates. 2nd Edn., Chapman and Hall/CRC, Boca Raton.

Da Silva, L.H.M. and A.J.A. Meirelles, 2000a. Phase equilibrium in polyethylene glycol/maltodextrin aqueous two-phase systems. Carbohydrate Polymers, 42: 273-278.

Da Silva, L.H.M. and A.J.A. Meirelles, 2000b. Bovine serum albumin, α-lactoalbumin and β-lactoglobulin partitioning in polyethylene glycol/maltodextrin aqueous-two-phase systems. Carbohydrate Polymers, 42: 279-282.

De Belder, A.N., 1993. Dextran. In Industrial Gums: Polysaccharides and Their Derivative,. Whistler, R.L. and J.N. BeMiller (Eds.). Academic Press, New York, pp: 399-425.

Demchenko, A.P., O.I. Rusyn and E.A. Saburova, 1989. Kinetics of the lactate dehydrogenase reaction in high-viscosity media. Biochim. Biophys. Acta, 998: 196-203.

De Paula, R.C.M. and J.F. Rodrigues, 1995. Composition and rheological properties of cashew tree gum, the exudate polysaccharide from *Anacardium occidentale* L. Carbohydrate Polymers, 26: 177-181.

De Paula, R.C.M., F. Heatley and P.M. Budd, 1998. Characterization of *Anacardium occidentale* exudate polysaccharide. Polymer Intl., 45: 27-35.

De Pinto, G.L., M. Martinez, J.A. Mendoza, E. Ocando and C. Rivas, 1995. Comparison of three Anacardiaceae gum exudates. Biochem. Syst. Ecol., 23: 151-156.

Ghosh, S., R. Vijayalakshmi and T. Swaminathan, 2004. Evaluation of an alternative source of dextran as a phase forming polymer for aqueous two-phase extractive system. Biochem. Eng. J., 21: 241-252.

Ginther, C.L., 1979. Sporulation and the production of serine protease and cephmycin C by *Streptomyces-lactamdurans*. Antimicrobial Agents and Chemotherapy, 15: 522-526.

Gotlieb, K.F. and A. Capelle, 2005. Starch Derivatization: Fascinating and Unique Industrial Opportunities. Wageningen Academic Publishers, Wageningen.

Gündüz, U. and K. Korkmaz, 2000. Bovine serum albumin partitioning in an aqueous two-phase system: Effect of pH and sodium chloride concentration. J. Chromatogr. B, 743: 255-258.

Han, J.H. and C.H. Lee, 1997. Effects of salts and poly (ethylene glycol) palmitate on the partitioning of proteins and *Bacillus subtilis* neutral protease in aqueous two-phase systems. Colloids and Surfaces B: Biointerfaces, 9: 109-116.

Heinze, T.J. and W.G. Glasser, 1998. Cellulose Derivatives: Modification, Characterization and Nanostructures. ACS Symposium Series 668. American Chemical Society, Washington.

Kennedy, J.F., 1974. Chemically reactive derivatives of polysaccharides. Advances in Carbohydrate. Chem. Biochem., 29: 305-405.

Kennedy, J.F. and C.A. White, 1983. Bioactive Carbohydrates in Chemistry, Biochemistry and Biology. Ellis Horwood, Chichester.

Kennedy, J.F., 1988. Carbohydrate Chemistry. Oxford University Press, Oxford.

Kennedy, J.F., C.J. Knill and D.W. Taylor, 1995. Maltodextrins. In: Handbook of Starch Hydrolysis Products and their Derivatives, Kearsley, M.W. and S.Z. Dziedzic (Eds.). Blackie Academic and Professional, Glasgow, pp: 65-82.

Knill, C.J. and J.F. Kennedy, 2005. Starch: Commercial Sources and Derived Products. In: Polysaccharides: Structural Diversity and Functional Versatility, Dumitriu, S. (Ed.). 2nd Edn. Marcel Dekker, New York, pp: 605-624.

Kroner, K.H., H. Hustedt and M.R. Kula, 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., H. Hustedt and M.R. Kula, 1984. Extractive enzyme recovery: Economic considerations. Process Biochem., 19: 170-179.

Kula, M.R., 1989. Large Scale Protein Recovery Using Aqueous Phase Systems. In: Separation Using Aqueous Phase Systems, Fisher, D. and I.A. Sutherland (Eds.). Plenum Press, New York, pp: 295-297.

Laurent, T.C., 1971. Interaction between polysaccharides and other macromolecules-enzyme reactions in polymer media. Eur. J. Biochem., 21: 498-506.

Lin, D.Q., Y.T. Wu, L.H. Mei, Z.Q. Zhu and S.J. Yao, 2003. Modeling the protein partitioning in aqueous polymer two-phase systems: Influence of polymer concentration and molecular weight. Chem. Eng. Sci., 58: 2963-2972.

Lindeström-Lang, K.U. and J.A. Schellman, 1959. Protein Structure and Enzyme Activity. In: The Enzymes, Vol. 1, Boyer, P.D., H. Lardy and K. Myrback (Eds.). 2nd Edn.. Academic Press, New York, pp: 443-510.

Marques, M.R. and J. Xavier-Filho, 1991. Enzymatic and inhibitory activities of cashew tree gum exudate. Phytochemistry, 30: 1431-1433.

Medin, A.S. and J.C. Janson, 1993. Studies on aqueous polymer two-phase systems containing agarose. Carbohydrate Polymers, 22: 127-136.

Medina-Torres, L., E. Brito-de la Fuente, B. Torrestiana-Sanchez and R. Katthain, 2000. Rheological properties of the mucilage gum (*Opuntia ficus indica*). Food Hydrocolloids, 14: 417-424.

Menestrina, J.M., M. Iacomini, C. Jones and P.A.J. Gorin, 1998. Similarity of monosaccharide, oligosaccharide and polysaccharide structures in gum exudate of *Anacardium occidentale*. Phytochemistry, 47: 715-721.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.

Mothé, C.G. and M.A. Rao, 1999. Rheological behavior of aqueous dispersions of cashew gum and gum arabic: Effect of concentration and blending. Food Hydrocolloids, 13: 501-506.

Mothé, C.G. and M.A. Rao, 2000. Thermal behavior of gum arabic in comparison with cashew gum. Thermochimica Acta, 357-358: 9-13.

Nair, G.M., K. Venkaiah and J.J. Shah, 1983. Ultrastructure of gum-resin ducts in cashew (*Anacardium occidentale*). Ann. Bot., 51: 297-305.

Nguyen, A.L., S. Grothe and J.H.T. Luong, 1988. Applications of pullulan in aqueous two-phase systems for enzyme production, purification and utilisation. Applied Biochem. Biotechnol., 27: 341-346.

Oliveira, L.A., L.A. Sarubbo, A.L.F. Porto, J.L. Lima-Filho, G.M. Campos-Takaki and E.B. Tambourgi, 2001. Physical and rheological characterisation of polyethylene glycol-cashew-nut tree gum aqueous two-phase systems. J. Chromatogr., B, 766: 27-36.

Park, K.M. and N.S. Wang, 1991. α-Amylase fermentation with *Bacillus amyloliquefaciens* in an aqueous two-phase system. Biotechnol. Progress, 7: 439-444.

Queiroz, J.A., F.A.P. Garcia and J.M.S. Cabral, 1995. Partitioning of *Chromobacterium viscosum* lipases in aqueous two-phase systems. Bioseparation, 5: 307-311.

Sarubbo, L.A., L.A. Oliveira, A.L.F. Porto, H.S. Duarte, A.M.A. Carneiro-Leão, J.L. Lima-Filho, G.M. Campos-Takaki and E.B. Tambourgi, 2000. New aqueous two-phase system based on cashew-nut tree gum and poly (ethylene glycol). J. Chromatogr., B, 743: 79-84.

Schmidt, A.S., A.M. Ventom and J.A. Asenjo, 1994. Partitioning and purification of α-amylase in aqueous two-phase systems. Enzyme and Microbial Technology, 16: 131-142.

Silva, D.A., R.C.M. de Paula, J.P.A. Feitosa, A.C.F. de Brito, J.S. Maciel and H.C.B. Paula, 2004. Carboxymethylation of cashew tree exudate polysaccharide. Carbohydrate Polymers, 58: 163-171.

Silva, D.A., J.P.A. Feitosa, J.S. Maciel, H.C.B. Paula and R.C.M. de Paula, 2006. Characterization of crosslinked cashew gum derivatives. Carbohydrate Polymers (In Press).

Sinha, R., S.P. Singh, S. Ahmed and S.K. Garg, 1996. Partitioning of a *Bacillus* alkaline protease in aqueous two-phase systems. Bioresource Technol., 55: 163-166.

Skoog, B., 1979. Determination of polyethylene glycol-4000 and glycol-6000 in plasma-protein preparations. Vox Sanguinis, 37: 345-349.

Skuse, D.R., R. Norris-Jones, M. Yalpani and D.E. Brooks, 1992. Hydroxypropyl cellulose/poly (ethylene glycol)-co-poly(propylene glycol) aqueous two-phase systems: System characterization and partition of cells and proteins. Enzyme Microbial. Technol., 14: 785-790.

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

Szlag, D.C. and K.A. Giuliano, 1988. A low cost aqueous two-phase system for enzyme extraction. Biotechnol. Techniques, 2: 277-282.

Tjerneld, F., I. Persson, P.Å. Albertsson and B. Hahn-Hägerdal, 1985. Enzymatic hydrolysis of cellulose in aqueous two-phase systems. II. Semicontinuous conversion of a model substrate. Biotechnol. Bioeng., 27: 1044-1050.

Tjerneld, F., S. Berner, A. Cajarville and G. Johansson, 1986. New aqueous two-phase system based on hydroxypropyl starch useful in enzyme purification. Enzyme Microbial. Technol., 8: 417-423.

Tjerneld, F., G. Johansson and M. Joelsson, 1987a. Lactate dehydrogenase on a large scale. Biotechnol. Bioeng., 30: 514-520.

Tjerneld, F., G. Johansson and M. Joelsson, 1987b. Affinity liquid-liquid extraction of lactate dehydrogenase in large scale. Biotechnol. Bioeng., 30: 809-816.

Tjerneld, F., 1989. New Polymers for Aqueous Two-Phase Systems. In: Separation Using Aqueous Phase Systems, Fisher, D. and I.A. Sutherland (Eds.). Plenum Press, New York, pp: 429-438.

Tjerneld, F. and G. Johansson, 1990. Aqueous two-phase systems for biotechnical use. Bioseparation, 1: 255-263.

Trachtenberg, S. and A.M. Mayer, 1982. Biophysical properties of *Opuntia ficus indica* mucilage. Phytochemistry, 21: 2835-2843.

Trón, L., B. Somogyi, S. Papp and M. Novák, 1976. Effect of viscous media in enzymic action. Studia Biophysica, 60: 157-162.

Van der Sanden, M.J.T., K. Nagy, I. Semsei and I. Zsnagy, 1995. An *in vitro* model of aging: The influence of increasing physical density on enzyme activities of trypsin, xanthine oxidase and superoxide dismutase. Arch. Gerontol. Geriatrics, 20: 273-282.

Venâncio, A., J.A. Teixeira and M. Mota, 1993. Evaluation of crude hydroxypropyl starch as a bioseparation aqueous two-phase-forming polymer. Biotechnol. Progress, 9: 635-638.

Venâncio, A., C. Almeida, L. Domingues and J.A. Teixeira, 1995. Protein partition on a derivative guar gum based aqueous two-phase system. Bioseparation, 5: 253-258.

Venâncio, A., C. Almeida and J.A. Teixeira, 1996. Enzyme purification with aqueous two-phase systems: Comparison between systems composed of pure polymers and systems composed of crude polymers. J. Chromatogr., B, 680: 131-136.

Veide, A., A.L. Smeds and S.O. Enfors, 1983. A process for large scale isolation of β-galactosidase from *E. coli* in an aqueous two-phase system. Biotechnol. Bioeng., 25: 1789-1800.

Vernau, J. and M.R. Kula, 1990. Extraction of proteins from biological raw material using aqueous polyethylene glycol-citrate phase systems. Biotechnology and Applied Biochem., 12: 397-404.

Villain-Simonnet, A., M. Milas and M. Rinaudo, 1999. Comparison between the physicochemical behaviour of two microbial polysaccharides: RMDP17 and rhamsan. Intl. J. Biolog. Macromolecules, 26: 55-62.

Villain-Simonnet, A., M. Milas and M. Rinaudo, 2000. A new bacterial polysaccharide (YAS34). I. Characterization of the conformations and conformational transition. Intl. J. Biol. Macromolecules, 27: 65-75.

Walter, H., I.E. Brooks and D. Fisher, 1985. Partitioning in Aqueous Two-phase Systems: Theory, Methods, Uses and Applications to Biotechnology. Academic Press, London.

Walter, H. and G. Johansson, 1986. Partitioning in aqueous two-phase systems: An overview. Anal. Biochem., 155: 215-242.

White, C.A. and J.F. Kennedy, 1981. Manual and automated spectrophotometric techniques for the detection and assay of carbohydrates and related molecules. Techniques in Carbohydrate Metabolism, B312: 1-64.

Wu, Y.T., M. Pereira, A. Venâncio and J. Teixeira, 2001. Separation of endo-polygalacturonase using aqueous two-phase partitioning. J. Chromatogr. A, 929: 23-29.

Yuryev, V.P., A. Cesàro and W.J. Bergthaller, 2002. Starch and Starch Containing Origins: Structure, Properties and New Technologies. Nova Science Publishers, Inc., New York.

Zakaria, M.B. and Z.A. Rahman, 1996. Rheological properties of cashew gum. Carbohydrate Polymers, 29: 25-27.

Zaslavsky, B.Y., 1995. Aqueous Two-Phase Partitioning, Physical Chemistry and Bioanalytical Applications, Marcel Dekker, New York.