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Phase equilibrium and protein partitioning in aqueous mixtures of maltodextrin with polypropylene glycol

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Abstract

Equilibrium data were obtained for polypropylene glycol, PPG (400 and 3500)/maltodextrin, MD (1000 and 2000) aqueous two-phase systems at 25°C. Four tie lines were measured for each system. The MD concentration in top phase is very small, and in some cases MD is almost excluded from this phase. The non random two-liquid (NRTL) model was used to correlate the equilibrium data. The partitioning behaviour of bovine serum albumin (BSA), α-lactoalbumin (α-La) and β-lactoglobulin (β-Lg) was studied in PPG 400/MD systems at 25°C. For most cases the proteins partition preferentially to the top phase.

Keywords: Aqueous two-phase systems; Maltodextrin; Polypropylene glycol; Non random two-liquid; Partitioning; Protein

1. Introduction

Aqueous two-phase systems formed by mixtures of two polymers, or one polymer and inorganic salts, are important for separation and purification of enzymes, proteins, nucleic acids, and other substances in biological processes (Albertsson, 1986; Vernau & Kula, 1990). This technology offers the advantages of high capacity, high activity yields and is easy to scale up. For large-scale processes, methods for recycling chemicals have been developed (Greve & Kula, 1991; Hustedt, 1986). Many phase diagrams for polyethylene glycol (PEG)/salt and PEG/dextran aqueous two-phase systems have been reported (Snyder, Cole & Szlag, 1992; Zaslavsky, 1995), while liquid–liquid equilibrium data on two-phase systems containing polypropylene glycol (PPG) are scarce, thus limiting the potential application of this specific system to biotechnology.

The most common polymer/polymer system is composed of dextran and PEG, but this system is very expensive for scaling up. This problem can be solved by the use of alternative polymers (Atkinson & Johns, 1994; Christian, Manley-Harris & Richards, 1998; Szlag, Giuliano & Snyder, 1990). Maltodextrin (MD) is a low-cost starch derivative that can be used as replacement for dextran in aqueous two-phase systems. Moreover, PPG is a polymer that is structurally closely related to PEG. PPGs of low molecular weights are soluble in water, while high molecular weight ones are only partially soluble (Molyneux, 1983).

Many authors have described the liquid–liquid equilibrium in aqueous two-phase systems (Kang & Sandler, 1987; Wu, Zhu, Lin & Mei, 1996; Wu, Lin & Zhu, 1998; Wu, Zhu, Lin & Lian, 1999) utilising a thermodynamic model. When equilibrium data are not available these models are utilised to provide the basis for extrapolating experimental data and predicting phase compositions. Furthermore, phase diagram data are necessary for the development of models that can predict phase separation.

In the present work, we report liquid–liquid equilibrium data for aqueous mixtures of MD (1000 and 2000) and PPG (400 and 3500) and the partition coefficients of the bovine serum albumin (BSA), α-lactoalbumin (α-La) and β-lactoglobulin (β-Lg) in PPG 400/MD at 25°C. The non random two-liquid (NRTL) model was used to correlate the equilibrium data for PPG/MD systems.

2. Experimental

2.1. Materials

PPG samples, with molecular weights of 400 and 3500, were purchased from Aldrich. MD 1000 and 2000 were supplied as courtesy by Companhia Lorenz (Blumenau, SC, Brazil). MD is a commercial polymer and therefore can be highly polydisperse. For this reason, this polymer

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Nomenclature

A	Parameter of NRTL model
C	Number of components
G	Parameter of NRTL model
M	Molecular weight
N	Total number of tie lines in a given group of data
T	Absolute Temperature (K)
w	Weight fraction
Superscripts/subscripts	
calc	Calculated data
ex	Experimental data
i, j, k	Components
I, II	Phases
n	Tie lines
Greek letters	
α	NRTL parameter
γ	Activity coefficient
τ	NRTL parameter

was analysed by gel permeation chromatography (GPC) in a Waters chromatograph (USA). The polydispersity indexes (M_w/M_n) of MDs 1000 and 2000 were found to be 1.22 and 1.74, respectively. The water content of each polymer was determined through Karl Fisher titration using a Metrohm equipment (Swiss). The water content was taken into account for preparing the MD stock solutions. The proteins BSA (96–99%), α-La and β-Lg from bovine milk, electrophoresis grade, were purchased from Sigma.

2.2. Aqueous two-phase equilibrium experiments

Centrifuge tubes, volume 15 cm^3, were used to carry out the phase equilibrium determinations. A stock solution containing 55% of MD was prepared by the addition of Milli Q water. PPG is liquid at ambient temperature and thus was utilised neat. For the determination of the tie lines, mixtures of appropriate amounts of MD stock solution, PPG and water were prepared on an analytic balance (A200 S Sartorius, Germany), with an accuracy in weight measurements of 0.0001 g. The systems were mixed for 10 min and then centrifuged (BR4i model, Jouan, France) at 2900g for 40 min at 25°C. The tubes were brought to equilibrium in a thermostatic bath (Viscotherm VT2, Physica, Germany) at 25°C ±0.1 for 5 h, since earlier tests indicated that this period of time was sufficient to ensure equilibrium. After this treatment, the two phases became clear and transparent, and the interface was well defined. After equilibrium was achieved, both phases were sampled with syringes. The top phase was sampled first, with care being taken to leave a layer of material at least 0.5 cm thick above the interface. The bottom phase was withdrawn using a syringe with a long needle.

2.3. Protein partitioning

Proteins were dissolved in the MD stock solution. Mixtures of known weights of MD stock solution, PPG and water were made up to a final mass of 12 g. The same procedure utilised to determine the equilibrium data was adopted to determine the partition coefficients. All systems contained nearly 50 mg (accurately weighed) of the selected

Table 1
Phase concentrations for PPG 400/MD systems

System	Total concentrations (wt%)			Top phase (wt%)			Bottom phase (wt%)		
	MD	PPG	Water	MD	PPG	Water	MD	PPG	Water
PPG 400/MD 1000	16.39	39.59	44.02	1.72	62.82	35.46	32.65	16.40	50.95
	22.50	30.70	46.80	1.88	62.54	35.58	33.73	15.54	50.73
	30.11	26.39	43.50	1.20	67.11	31.69	42.78	11.83	45.36
	30.59	33.61	35.80	0.34	75.94	23.72	52.70	7.20	40.10
PPG 400/MD 2000	26.69	22.10	51.21	11.30	37.32	51.38	33.43	13.88	52.69
	28.45	24.62	46.93	4.75	52.04	43.21	42.41	11.07	46.52
	29.94	28.45	41.61	1.86	64.06	34.08	47.17	9.13	43.70
	31.15	32.45	36.40	0.95	71.93	27.12	51.93	7.11	40.96

Table 2
Phase concentrations for PPG 3500/MD systems

System	Total concentrations (wt%)			Top phase (wt%)			Bottom phase (wt%)		
	MD	PPG	Water	MD	PPG	Water	MD	PPG	Water
PPG 3500/MD 1000	16.43	40.07	43.50	0.00	96.59	3.41	27.69	1.74	70.57
	22.51	30.68	46.81	0.00	97.12	2.88	32.60	2.05	65.35
	29.96	26.66	43.38	0.00	96.27	3.73	40.98	2.47	56.55
	32.91	28.57	38.52	0.00	96.56	3.44	46.76	2.80	50.86
PPG 3500/MD 2000	26.72	22.03	51.25	0.06	91.59	8.35	34.34	1.62	64.04
	28.71	24.97	46.32	0.02	93.51	6.47	38.48	1.64	59.88
	29.90	28.56	41.54	0.01	96.76	3.23	42.52	1.66	55.82
	31.07	32.57	36.36	0.01	97.84	2.15	46.76	1.82	51.42

protein. Visual estimates of the volumes of top and bottom phases were made in graduated centrifuge tubes. The volume of phases were then used to estimate the volume ratio (V_r = volume of top phase/volume of bottom phase).

2.4. Analytical methods

The concentration of MD was determined by polarimetry (Carl Zeiss Jena, POLAMAT A model, equipped with a mercury lamp at 546 nm, Germany). The standard deviation of the weight percent of MD by this method was ±0.009%.

The concentration of water was determined by freeze-drying (EZ DRY model, FTS Systems, New York, USA) at −54°C and 100 mTorr for 48 h. The standard deviation of the weight percent of water by this method was ±0.004%.

The concentration of PPG was determined by difference. The standard deviation of the weight percent of PPG was ±0.006%.

The concentrations of proteins (top and bottom) were determined by spectrophotometry. A sample of each phase was mixed with distilled water and its absorbance read at 280 nm using a spectrophotometer (Hach DR-4000U). In all cases the concentrations were analysed in triplicate. The mean standard deviation for protein concentration was ±0.0007 mg/g. The partition coefficient (K) was calculated as $K_{Protein} = C_T/C_B$, where C_T and C_B are the protein concentrations in mg/g of the top and bottom phases, respectively. For the partition coefficients the standard deviation was ±0.001.

3. Results

3.1. Liquid–liquid equilibrium

The experimental liquid–liquid equilibrium results for the aqueous two-phase systems PPG 400/MD and PPG 3500/MD are given in Tables 1 and 2. All the results are expressed as the weight percentage. For each polymer combination, four tie lines were determined.

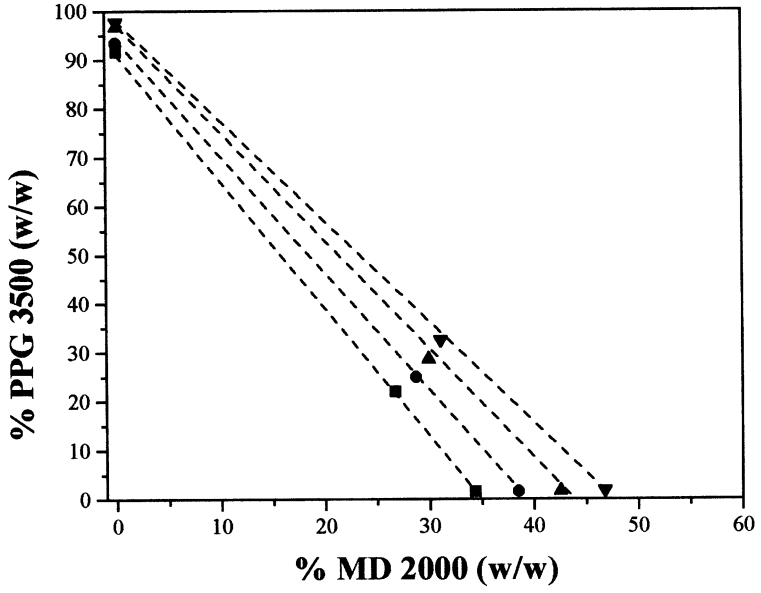

Fig. 1. Experimental tie lines for the system PPG 3500/MD 2000 at 25°C.

Table 3
Tie line lengths, volume ratios and partition coefficients of BSA, α-La and β-Lg in PPG/MD systems

System PPG/MD	Total concentrations (%PPG/MD)	TLL (%w/w)	V_r	K_{BSA}	$K_{\alpha\text{-La}}$	$K_{\beta\text{-Lg}}$
400/1000	25.78/33.16	69.41	1.52	3.22	1.73	1.56
	27.71/32.78	70.02	1.56	1.99	1.33	1.25
	30.60/33.50	78.57	1.73	1.50	1.09	0.86
	32.69/33.63	92.15	1.80	1.45	1.17	0.90
400/2000	22.14/28.82	43.38	2.34	1.88	1.70	1.12
	24.92/30.83	64.24	1.86	2.66	2.15	1.89
	27.94/31.82	78.46	1.80	2.06	3.28	3.01
	30.48/34.75	88.16	1.78	2.17	1.54	0.75

For most systems the MD concentration in top phase is very small, and in some cases MD is almost excluded from this phase. For the system PPG 3500/MD this trend is even more pronounced (Fig. 1). Similar results were reported by Zafarani-Moattar and Salabat (1998) for PPG/Salt systems. All PPG/MD systems were characterised by the presence of considerable quantities of PPG in the top phase. This quantity increased markedly as the molecular weight of PPG increased. The same behaviour was observed by Cheluget, Gelinas, Vera and Weber (1994) in PEG/salt systems. In all systems studied in the present work, the PPG concentration in top phase is relatively high when compared to systems formed with PEG/dextran, PEG/salt or PEG/MD (Albertsson, 1986; Silva, Coimbra & Meirelles, 1997; Silva & Meirelles, 2000a; Zaslavsky, 1995). The systems PPG/MD required particularly large concentrations of both polymers to exhibit phase splitting. The very high total polymer concentration necessary for inducing phase separation is most likely due to the very low molecular weight of the polymers.

The commercial MD samples used in the present work were produced by a combination of both acid and enzymatic hydrolysis of starch. Such processing strategy reduces the tendency to retrogradation (Atkinson & Johns, 1994). This process produces low molecular weight MD, which exhibits a distinct cost advantage over other specifically modified starch derivatives used to retard retrogradation.

The effect of increasing the molecular weight of PPG was to increase the region of two-phase coexistence, a behaviour already reported in the literature for similar systems (Albertsson, 1986; Cheluget et al., 1994; Szlag et al., 1990).

3.2. Protein partitioning

BSA, α-La and β-Lg were partitioned at 25°C in the PPG 400/MD systems. The partition coefficient results given in Table 3 show that for most cases the proteins partition preferentially to the top phase ($K_{Protein} > 1$), the PPG-rich phase. The partition coefficients obtained for the same proteins in PEG/MD systems (Silva & Meirelles, 2000b) are in most cases lower than the values reported in the present work.

In partitioning studies it is conventional to express the difference in the composition of the two-phases by the tie line length (TLL), which is determined by the difference in concentration of the system forming components, PPG and MD (Belval, Brenton, Huddleston & Lyddiatt, 1998; Forciniti, Hall & Kula, 1991). TLL, expressed in weight percentage of polymers, is calculated according to Eq. (1) given below:

$$\%\mathrm{TLL} = \sqrt{(w^{\mathrm{I}}_{\mathrm{PPG}} - w^{\mathrm{II}}_{\mathrm{PPG}})^2 + (w^{\mathrm{I}}_{\mathrm{MD}} - w^{\mathrm{II}}_{\mathrm{MD}})^2} \times 100 \qquad (1)$$

where w_{PPG} and w_{MD} are the weight fractions of PPG and MD, respectively, and I and II represent top and bottom phases, respectively.

Partitioning in PEG/Dextran system is usually carried out at TLLs between 10 and 30 (wt% polymer) while in PEG/MD system it is carried out at TLLs between 20 and 60 (wt% polymer) (Silva & Meirelles, 2000b). In the present work, we have obtained TLLs between 40 and 90 (wt% polymer) for the PPG/MD system.

The partition coefficients of α-La, β-Lg and BSA as function of TLL for the PPG 400/MD systems are shown in Figs. 2 and 3. In the system PPG 400/MD 1000 (Fig. 2), the partition coefficients decrease as the TLL increases. The same behaviour was obtained by Albertsson (1986) for serum albumin partitioned in PEG/dextran systems, Peng, Li and Li (1995) for lysozyme in PEG/phosphate potassium systems and Silva and Meirelles (2000b) for α-La in PEG/MD systems.

In PPG 400/MD 2000 the partition coefficients of α-La and β-Lg increase as TLL increases until a length of 78.5% (Fig. 3). This TLL corresponds to a water concentration of approximately 34% and a PPG concentration of 64% in the top phase. As shown in Table 1, for the system PPG 400/MD 1000 the PPG concentration in top phase is always higher than 62%, and its TLLs are generally larger than the corresponding ones for the PPG 400/MD 2000 system. It seems that for very high PPG concentration in the top phase, the proteins present there tend to move to the bottom phase, decreasing the partition coefficients. Silva and Meirelles (2000b) observed that the partition coefficients for BSA and β-Lg increase as TLL increases in PEG 8000/MD 2000 system. But for the PEG/MD systems the highest TLL was 61% and the PEG concentration in the top phase was always lower than 37%.

In PPG 400/MD 1000 system, an increase in the partition coefficients of proteins was accompanied by a decrease of the volume ratio, V_r (see Table 3). These results agree with those obtained by Huddleston, Abelaria, Wang and Lyddiatt (1996) in PEG/potassium phosphate systems and by Johansson, Karlström, Tjerneld and Haynes (1998) for several aqueous two-phase systems. For PPG 400/MD 2000 system we have observed an opposite behaviour, which is similar to

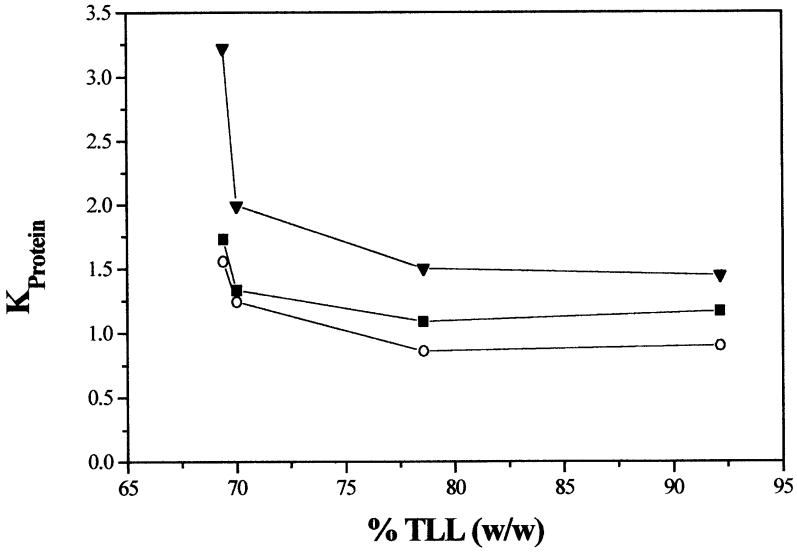

Fig. 2. Effect of tie line length on partition coefficients in PPG 400/MD 1000 system. BSA (▼), α-La (■), β-Lg (○).

that reported by Marcos, Fonseca, Ramalho and Cabral (1998) for *penicillin acylase* in PEG/sodium citrate systems.

4. Modelling

The NRTL equation was utilised for modelling the PPG/MD aqueous two-phase systems. Due to the large difference in molecular weights between the components in the systems, the weight fraction was used as unit of concentration. This procedure was suggested by Oishi and Prausnitz (1978) for the UNIQUAC and UNIFAC models.

Eq. (2) shows the NRTL model expressed in weight fraction:

$$\ln \gamma_i = \frac{\sum_j^C \frac{\tau_{ji} G_{ji} w_j}{M_j}}{\sum_j^C \frac{G_{ji} w_j}{M_j}} + \sum_{j=1}^C \left[\frac{w_j G_{ji}}{M_j \sum_k^n \frac{G_{kj} w_k}{M_k}} \left(\tau_{ij} - \frac{\sum_k^C \frac{\tau_{kj} G_{kj} w_k}{M_k}}{\sum_k^C \frac{G_{kj} w_k}{M_k}} \right) \right] \quad (2)$$

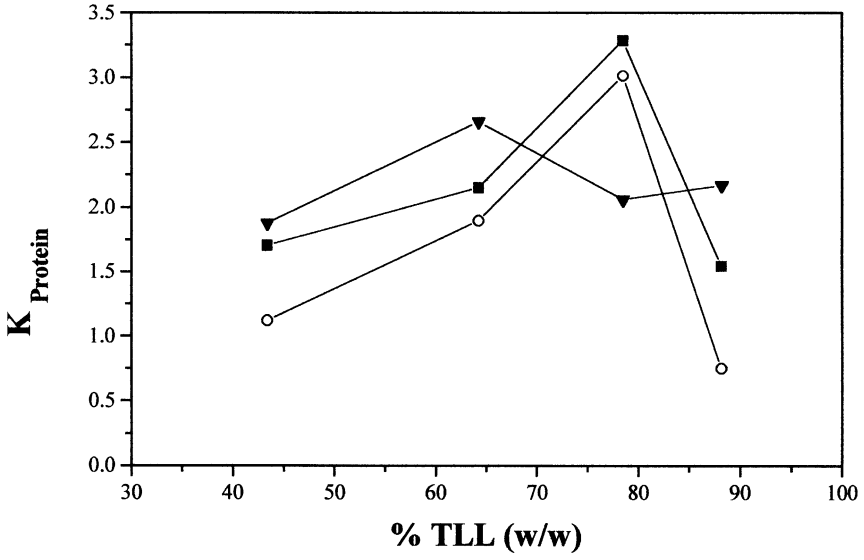

Fig. 3. Effect of tie line length on partition coefficients in PPG 400/MD 2000 system. BSA (▼), α-La (■), β-Lg (○).

Table 4
Adjusted parameters of the NRTL model

Parameters	A_{ij} (K)	A_{ji} (K)	$\alpha_{ij} = \alpha_{ji}$
12[a]	−375.35	937.93	0.3801
13	2986.50	544.31	0.2000
23	918.78	−1873.50	0.2884
15	2274.6	−25.01	0.0912
25	2681.30	−1240.30	0.4700
42	−349.20	2292.80	0.4699
43	30.85	−5000.00	0.4185
45	−38.58	1820.80	0.1999

[a] PPG 400 (1), Water (2), MD 1000 (3), PPG 3500 (4), MD 2000 (5).

where

$$G_{ij} = \exp(-\alpha_{ij}\tau_{ij}) \tag{3}$$

$$\tau_{ij} = A_{ij}/T \tag{4}$$

$$\alpha_{ij} = \alpha_{ji} \tag{5}$$

Following the procedure developed by Stragevitch and d'Ávila (1997), adjustments of the parameters were made by minimisation of the maximum likelihood objective function. Table 4 shows the parameters estimated from the experimental data. Figs. 4 and 5 show the experimental and calculated tie lines and the calculated binodal curves for the systems PPG 400/MD 1000 and PPG 400/MD 2000.

For these aqueous two-phase systems the correlation was successful. The experimental data were compared to the calculated values by liquid–liquid flash using the adjusted parameters. The deviation between the experimental and calculated weight fractions for each system is given in Table 5, calculated according to Eq. (6):

Table 5
Percent deviation of experimental to calculated weight fractions

PPG/MD system	Δw (%)
400/1000	0.38
400/2000	0.31
3500/1000	3.96
3500/2000	1.26
Average deviation	1.30

$$\Delta w = 100\sqrt{\frac{\sum_{n=1}^{N}\sum_{i=1}^{C}[(w_{n,i}^{\mathrm{I,ex}} - w_{n,i}^{\mathrm{I,calc}})^2 + (w_{n,i}^{\mathrm{II,ex}} - w_{n,i}^{\mathrm{II,calc}})]}{2NC}} \tag{6}$$

The best result was obtained for the system PPG 400/ MD 2000. For the system PPG 3500/MD 1000 the obtained result was not satisfactory if compared with the results for other systems. The low average deviation of the NRTL model for most MD/PPG/water systems shows that it is possible to obtain a significant set of parameters, which describes well such systems.

5. Conclusions

Equilibrium analysis of phase behaviour for MD/PPG/water systems were conducted at 25°C for different concentrations and molecular weights of both polymers. These systems were obtained by a combination of PPGs with

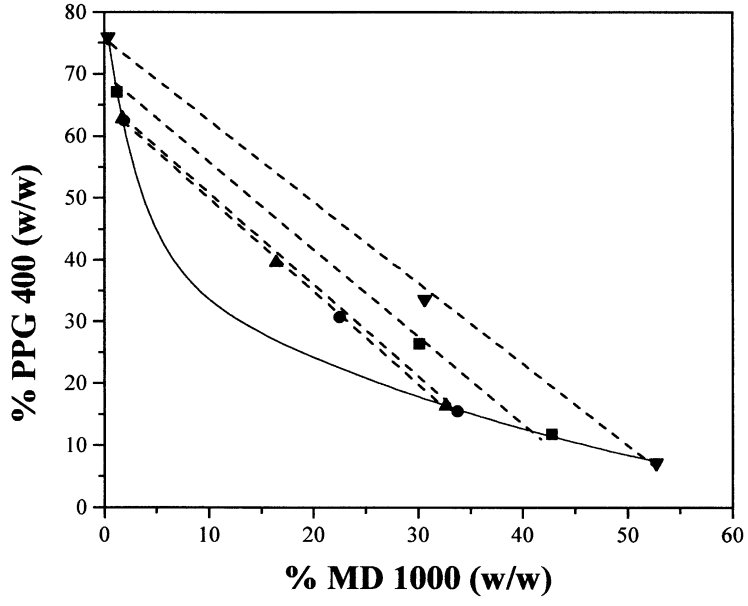

Fig. 4. Experimental (▲, ●, ■, ▼) and calculated tie lines (dashed ones) and the calculated binodal curve (continuous line) for the PPG 400/MD 1000 system.

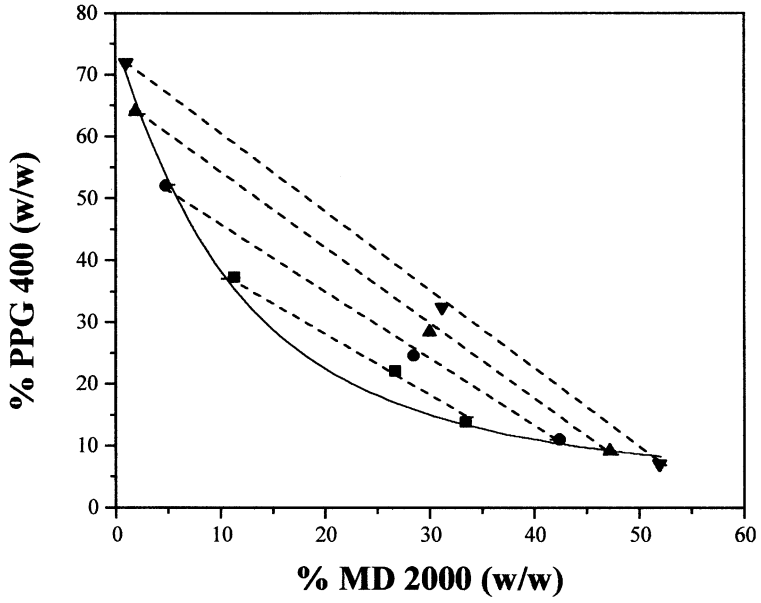

Fig. 5. Experimental (■, ●, ▲, ▼) and calculated tie lines (dashed ones) and the calculated binodal curve (continuous line) for the PPG 400/MD 2000 system.

molecular weights 400 and 3500 and MD with molecular weights 1000 and 2000. For these systems the MD concentration in top phase is very small, and in some cases MD is almost excluded from this phase. This effect is more pronounced in PPG 3500/MD systems. In all systems the PPG concentration in top phase is relatively high when compared to systems formed with PEG/dextran, PEG/MD or PEG/salt. A much higher concentration of MD is also required to form aqueous two-phase systems, as compared to dextran or salt.

BSA, α-La and β-Lg were partitioned at 25°C in the PPG 400/MD systems. For most cases the proteins partition preferentially to the top phase ($K_{\text{Protein}} > 1$), the PPG-rich phase. In PPG 400/MD 1000 system the partition coefficients of α-La, β-Lg and BSA decreases as the TLL increases. In this system, an increase in the partition coefficients of proteins was accompanied by a decrease of the volume ratio, V_r. The partition coefficients of α-La and β-Lg in PPG 400/MD 2000 increase as TLL increases until a length of 78.5%. For TLL higher than 78.5% this trend is inverted and the partition coefficients tend to decrease.

The adjustment of the NRTL model to the experimental data showed that it was possible to obtain a significant set of parameters, which well describes the equilibrium for MD/PPG/water systems.

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