Phase-separation-induced fractionation in molar mass in aqueous mixtures of gelatin and dextran

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An overview of the effects of phase separation of aqueous mixtures of gelatin and dextran on the fractionation in molar mass of these two components is given. Molar mass distributions in coexisting phases were investigated using size exclusion chromatography with multiangle laser light scattering. The initial molar mass of the native material, concentration, and temperature were varied. The results show a strong fractionation in molar mass for both components. The molar mass of the native material and concentration appeared to be the only factors that affected the final molar mass distributions, temperature having no effect. The results show that in the molar mass range where fractionation is the strongest, i.e., roughly below the maximum in the distribution, fractionation is governed by a Boltzmann factor $e^{-\Delta G/kT}$, where ΔG denotes the free energy involved in transferring a polymer with a certain length from the enriched to the depleted phase, and in this case turns out to be proportional to the molar mass. Comparison of the results of phase separation with results on dialysis shows that water affinity is not the driving force for the phase separation of gelatin and dextran in aqueous solution. The gelation properties of gelatin in both phases were also determined. The gelation properties of gelatin in the coexisting phases differ from those of native gelatin. In particular, the gelatin in the gelatin-poor phase shows strong differences compared to the native material.

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I. INTRODUCTION

At sufficient concentration (above 3 wt % of each component), segregative phase separation takes place in aqueous mixtures of gelatin and dextran. This phase separation results in a gelatin-rich and a dextran-rich phase. Such phase separation processes are often the basis for structuring processed foods. In the literature, a large quantity of experimental data is available on the compatibility of food biopolymers [1]. Often biopolymers, certainly extracellular bacterial and cell-wall biopolymers, used in food products are polydisperse in their molar mass. Due to this polydispersity, fractionation in molar mass takes place during phase separation. This results in different molar mass distributions in the coexisting phases relative to the original mixture. The denser phase favors larger particles [2]. Fractionation was studied in the past, e.g., in emulsions [3] and in mixtures of β-lactoglobulin aggregates, and κ-carrageenan [4]. However, quantitative theories only exist for a narrow molar mass distribution [5].

In spite of the presence of fractionation, in several studies the mechanical properties of the mixed gels are compared with [6–9] or even calculated with [10] the properties of the native material. We show that the functional properties, such as gel strength and specific viscosity, of the material in both phases differ from each other and from the native material.

The work presented here gives an insight into the fractionation of the molar mass due to phase separation of aqueous mixtures of gelatin and dextran in different conditions. The initial molar mass distributions, concentration, and equilibrium temperature were varied. The system gelatin/dextran was chosen because of its good experimental accessibility. We note that in this paper phase separation always took place at temperatures above the gelation temperature of gelatin ($\approx$30–35 °C), in order to enable full equilibrium phase separation. So in this paper we do not consider cases of phase separation in conjunction with gelation.

The role of the low molecular mass part of the distribution in the establishment of an osmotic pressure equilibrium was studied using dialysis experiments, in which the phase separation was imposed by a semipermeable membrane.

II. EXPERIMENT

A. Materials

Porcine skin gelatin (ISO electric point $\pm$8.7, bloom 305 g, $M_w \approx 170$ kDa) was kindly provided by Degussa Biosystems (Center de Recherches, 50500 Baupte, France). Gelatin samples with M_w of 43 kDa (bloom 93 g) and 74 kDa (bloom 281 g) were kindly provided by DGF Stoess, Germany. Dextran with M_w of 148 and 282 kDa was purchased from Sigma Chemicals. The ingredients were used without further purification. Solutions were prepared by gravimetrically adding solvent (0.1M NaCl and 0.02% NaN_3 to prevent bacterial growth) to the proper amount of material. Dextran dissolves readily at room temperature. Gelatin was dissolved by stirring the mixture overnight on a magnetic stirrer at approximately 50 °C.

B. Determination of the temperature-composition phase diagram

To determine the temperature-composition phase diagram, mixtures of equal weight concentrations of gelatin and dex-

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tran in 0.1*M* NaCl were made. For this purpose two equal weights of a dextran solution and a gelatin solution with the same weight percentage polymer were mixed. Approximately 10 g of the mixture was put into a plastic tube. To prevent evaporation of the solvent, paraffin oil was added on the top of the meniscus. The tubes were held in a water bath at a temperature above the gel temperature of gelatin for approximately 20 h to reach equilibrium of the phase separation. Equilibrium was assumed to be reached when a sharp meniscus between transparent gelatin-rich and dextran-rich fluid layers was observed. Hereafter, the heights of the gelatin-rich and dextran-rich phases were measured to calculate the volume of the phases. Samples of these phases were taken with a syringe with hypodermic needle.

To determine the concentrations of gelatin and dextran in the coexisting phases, samples taken from the gelatin-rich and dextran-rich phases were diluted 40 times in 0.1*M* NaCl. Optical rotation was measured at two different wavelengths (365 and 578 nm) at 80 °C. With the use of calibration lines, the concentrations in the diluted phases were calculated by solving the system

$$\alpha_{\mathrm{meas}}(c_g, c_d, \lambda = 365\ \mathrm{nm}) = [\alpha]_{g,\lambda=365\ \mathrm{nm}} c_g + [\alpha]_{d,\lambda=365\ \mathrm{nm}} c_d,$$

$$\alpha_{\mathrm{meas}}(c_g, c_d, \lambda = 578\ \mathrm{nm}) = [\alpha]_{g,\lambda=578\ \mathrm{nm}} c_g + [\alpha]_{d,\lambda=578\ \mathrm{nm}} c_d, \qquad (1)$$

where the subscripts ''meas,'' *g*, and *d* mean measured, gelatin, and dextran. $[\alpha]$ is the specific rotation (per wt %) at a definite wavelength. Calibration lines to obtain $[\alpha]_g$ and $[\alpha]_d$ were obtained in the range from 0 to 1 wt % at 80 °C and at the two different wavelengths. This method is valid only if gelatin and dextran contribute to the optical rotation proportionally to their concentration. To probe this simple additivity of the contributions to the optical rotation in a mixture, for each wavelength the optical rotation of five symmetrical mixtures (i.e., solute consisting of 50% gelatin and 50% dextran) of gelatin and dextran was measured and compared with that of the sum of the pure components. The results were in satisfactory agreement with the assumption of additivity.

The weight of the phases was derived from the height of the phases. To calculate the weight fraction of a phase, the approximation was made that both phases had the same density.

C. Dialysis of gelatin solution against dextran solution

Dialysis tubing with a pore size of 12–14 kDa was first boiled in water. Two solutions were made, one of 8.0 wt % gelatin in 0.1*M* NaCl and one of 8.0 wt % dextran (282 kDa) in 0.1*M* NaCl. 10 g of the gelatin solution was put in the dialysis tubing. This tubing was immersed in 500 g of the dextran solution for 20 h. The gelatin as well as the dextran concentrations were measured.

D. Determination of the molar mass distribution of gelatin and dextran

Size exclusion chromatography (SEC) equipped with a multiangle laser light scattering (MALLS) detector and a refractive index (RI) detector was used to determine the molar mass distribution of gelatin and dextran. For mixtures of gelatin and dextran, an additional detector monitoring optical rotation (OR) was applied at 365 nm. By combining the signals from the RI detector and the OR detector, the contribution from dextran and gelatin to the two signals can be unraveled using the set of equations in Eq. (1). The only difference is that $[\alpha]_g$ and $[\alpha]_d$ at $\lambda = 578$ nm were replaced by the refractive index increments of, respectively, gelatin and dextran. Thus,

$$\alpha_{\mathrm{meas}}(c_g, c_d, \lambda = 365\ \mathrm{cm}) = [\alpha]_{g,\lambda=365\ \mathrm{nm}} c_g + [\alpha]_{d,\lambda=365\ \mathrm{nm}} c_d,$$

$$\Delta n_{\mathrm{meas}}(c_g, c_d) = \left(\frac{dn}{dc_g}\right) c_g + \left(\frac{dn}{dc_d}\right) c_d, \qquad (2)$$

with Δn the difference relative to the buffer solution and dn/dc_i the refractive index increment due to concentration c_i of species *i*. The values of dn/dc_i in the buffer used were 0.159 for gelatin and 0.130 for dextran.

A $LiNO_3/KH_2PO_4/K_2HPO_4$ (*p*H 6.7) buffer was used as eluant. The flow rate was 1 ml min^{-1}. The columns that were used were a combination of TSK guard + TSK G5000PW + TSK G3000PW. Typically 4 mg of dry material in 200 μl was injected, resulting in a concentration of 0.2 wt % in the detector cells.

For the determination of the molar mass distribution of gelatin and dextran, samples were taken from the gelatin-rich and dextran-rich phases after equilibration of ca. 20 h (see Table I). In addition to these mixtures, the pure components were also determined. All samples from the coexisting phases were diluted 40 times in the eluant and put in vials. Before detection, the vials were stored for 30 min at 80 °C. The temperature of the SEC column and the MALLS detector was 50 °C. The OR detector cell had a temperature of 40 °C and the RI detector cell was not temperature controlled.

The expressions used to calculate the molar mass averages are the following:

$$M_n = \frac{\sum_i c_i}{\sum_i (c_i/m_i)}, \qquad (3)$$

$$M_w = \frac{\sum_i c_i m_i}{\sum_i c_i}, \qquad (4)$$

TABLE I. Overview of the samples studied (concentrations in wt %, temperature in °C).

Sample code	Conc. gelatin 170 kDa	Conc. gelatin 43 kDa	Conc. gelatin 74 kDa	Conc. dextran 148 kDa	Conc. dextran 282 kDa	T_{PS}[a]
gel 170	5.0					
mix gel		2.5	2.5			
dex 148				5.0		
dex 282					5.0	
mix dex				2.5	2.5	
*A*1	5.0				5.0	50
*A*2	5.0				5.0	60
*A*3	5.0				5.0	70
B	4.5				4.5	60
C	4.0				4.0	60
D	5.0			5.0		60
E	5.0			2.5	2.5	60
F		2.5	2.5		5.0	60

[a]T_{PS} is the temperature at which phase separation was made to take place.

$$M_z = \frac{\sum_i c_i m_i^2}{\sum_i c_i m_i}, \tag{5}$$

with c_i (g ml^{-1}) the concentration of molecules with molar mass m_i. The polydispersity is defined as M_w/M_n.

E. Determination of the mechanical properties of gelatin

In order to measure the mechanical properties of gelatin in the two phases, gelatin has to be extracted from these phases. For this purpose a mixture of 5.0 wt % gelatin (M_w 170 kDa) and 5.0 wt % dextran (M_w 282 kDa) in 0.1*M* NaCl was left to phase separate for 20 h at 60 °C. Samples of the upper and lower phases were taken. The sample of the dextran-rich phase was cooled down so that phase separation continued and gelled droplets of gelatin were formed. This phase was centrifuged (60 min, 12 000*g*) until the gelled gelatin droplets had formed a precipitate. The concentrations of gelatin and dextran in this precipitate and in the upper phase were measured using polarimetry. Both contained less than 1 wt % dextran. The upper phase as well as the precipitate were diluted with 0.1*M* NaCl to a gelatin concentration of 2 wt %. A solution of 10 wt % native gelatin in 0.1*M* NaCl was made and stored for 20 h in a water bath at 60 °C. This solution was also diluted to a concentration of 2% gelatin. Small-deformation oscillatory measurements were performed on a strain controlled rheometer (Rheometrics, Fluids Spectrometer RFS II) using a cone and bob system at 1% strain and 1 Hz. First, the gelatin solution was cooled from 50 to 15 °C. After that, the temperature was held at 15 °C for 1 h and the aging of the gel was followed. Finally a strain sweep was performed between 1 and 100 % strain.

III. RESULTS

A. Phase diagram

Figure 1 is the temperature-composition phase diagram of mixtures containing 5.0 wt % gelatin and 5.0 wt % dextran, for dextran molar masses of 148 and 282 kDa. There turns out to be no significant effect of either the temperature or the dextran molar mass on the phase composition in the temperature region that was studied. It is expected that lowering the initial total polymer concentration will bring the system closer to the miscible state. In other words, by diluting the system, the phase compositions should become more similar. This is indeed observed in Fig. 2. In this figure is also indicated the only composition [(3.5 wt %)/(3.5 wt %)] for which a temperature-induced phase transition could be observed in the temperature range between the gelation of gelatin (ca. 30 °C) and 80 °C, above which gelatin decomposes.

Figure 3 shows the composition-composition phase diagram for a range of temperatures above the gelation tempera-

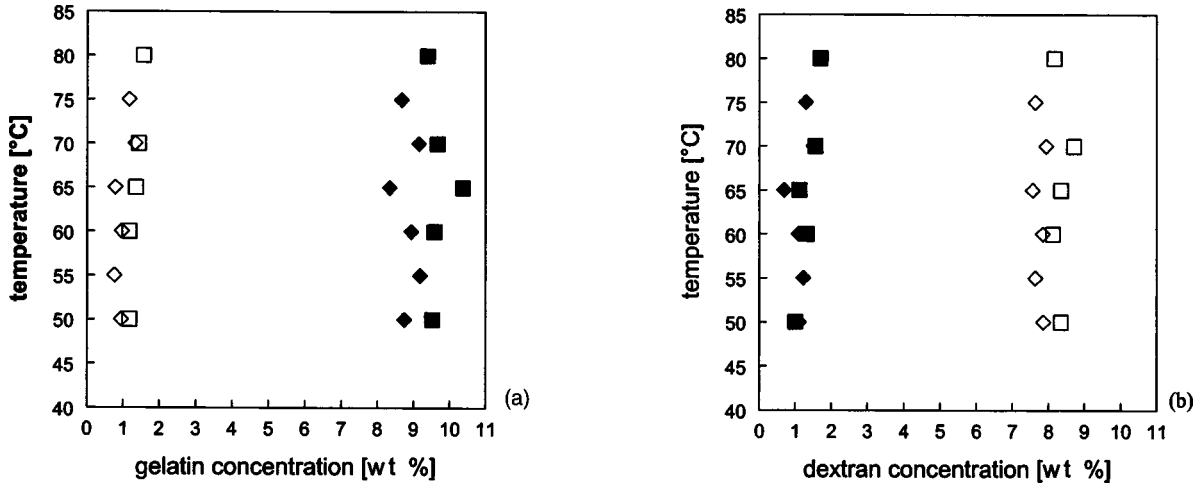

FIG. 1. Coexisting phase compositions in gelatin/dextran/0.1*M* NaCl for M_w of dextran 148 kDa and dextran 282 kDa. (a) Gelatin concentrations, (b) dextran concentrations. Initial compositions of gelatin/dextran: 5 wt %/5 wt %. ■, □, dextran 148 kDa; ◆, ◇, dextran 282 kDa. Open symbols represent dextran-rich phase, closed symbols represent gelatin-rich phase.

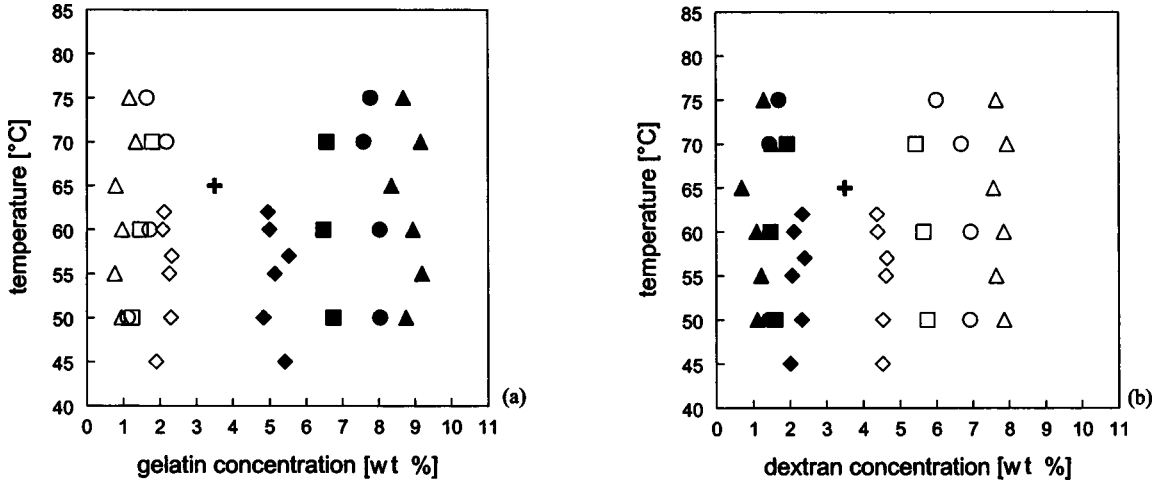

FIG. 2. Coexisting phase compositions in gelatin/dextran/0.1*M* NaCl for M_w of dextran 282 kDa. (a) Gelatin concentrations, (b) dextran concentrations. Initial compositions of gelatin/dextran: ◆, ◇, 3.5%/3.5%; ■, □, 4%/4%; ●, ○, 4.5%/4.5%; ▲, △, 5%/5% (all percentages w/w). Open symbols represent dextran-rich phase, closed symbols represent gelatin-rich phase. +, temperature where the mixture 3.5% gelatin and 3.5% dextran no longer showed phase separation.

ture of gelatin. This figure also shows the temperature independence of the phase diagram.

B. Molar mass distributions

An overview of the molar masses of the pure components is given in Table II. Figures 4–7 show the molar mass distributions of gelatin and dextran in coexisting phases, as well as the distributions prior to phase separation. The curves are normalized to the concentration of the original sample (using the elution volume as the quantity on the horizontal axis), i.e., the areas under the curves are equal to the concentrations in the coexisting phases. The area under the curves prior to phase separation of gelatin and dextran is 5.0 wt %, i.e., the overall concentration in the phase separating mixture. From the SEC MALLS data for the pure components, the elution volume was converted to the molar mass of each component. The lowest molar mass which could be reliably detected was 40 kDa.

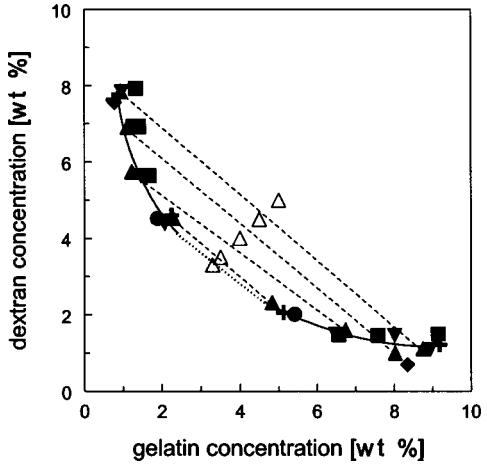

FIG. 3. Composition-composition phase diagram of gelatin and dextran 282 kDa in 0.1*M* NaCl. The closed symbols represent the coexisting phases at ●, 45; ▲, 50; +, 55; ▼, 60; ◆, 65; ■, 70 °C. The open triangles represent the initial mixtures at 50 °C.

As can be seen in these figures, both the gelatin and dextran are being fractionated by the phase separation. The molar mass of each component in each phase is calculated [see Tables III(a) and III(b)]. In these tables the polydispersity of the components is also given.

1. Molar mass effects

Comparing the phase diagrams in which the molar mass of dextran is varied (Fig. 1), it can be concluded that the molar mass of dextran hardly influences the composition of the phases. Figure 4 shows the influence of the molar mass of dextran on the molar mass distribution of the two components in the two phases.

Let us first look at the results for gelatin in both phases [Fig. 4(a), Table III(a)]. Figure 4(a) shows that the molar mass of dextran has no influence on the distribution of gelatin in both phases. From the data for the gelatin-rich phase in Table III(a) the same conclusion can be drawn.

The results for dextran in the dextran-rich phase show a downward shift of the molar mass distribution and the average molar mass values with decreasing initial molar mass of dextran. The opposite happens in the dextran-poor phase: all values of dextran increase with decreasing initial dextran molar mass. In Table III(b) it can be seen that the average molar mass values of the mixture of dextran in sample *E* lie between the values of samples *A*2 (dextran 282 kDa) and *D* (dextran 148 kDa).

TABLE II. M_n, M_w, and M_z and the polydispersity for gelatin and dextran before phase separation (molar mass averages in kDa).

	M_n	M_w	M_s	M_w/M_n
Gelatin 170 kDa	97	184	422	1.9
Gelatin 43 kDa+74 kDa	69	147	379	2.1
Dextran 148 kDa	60	146	391	2.4
Dextran 282 kDa	64	299	993	4.7
Dextran 148 kDa+282 kDa	63	261	915	4.1

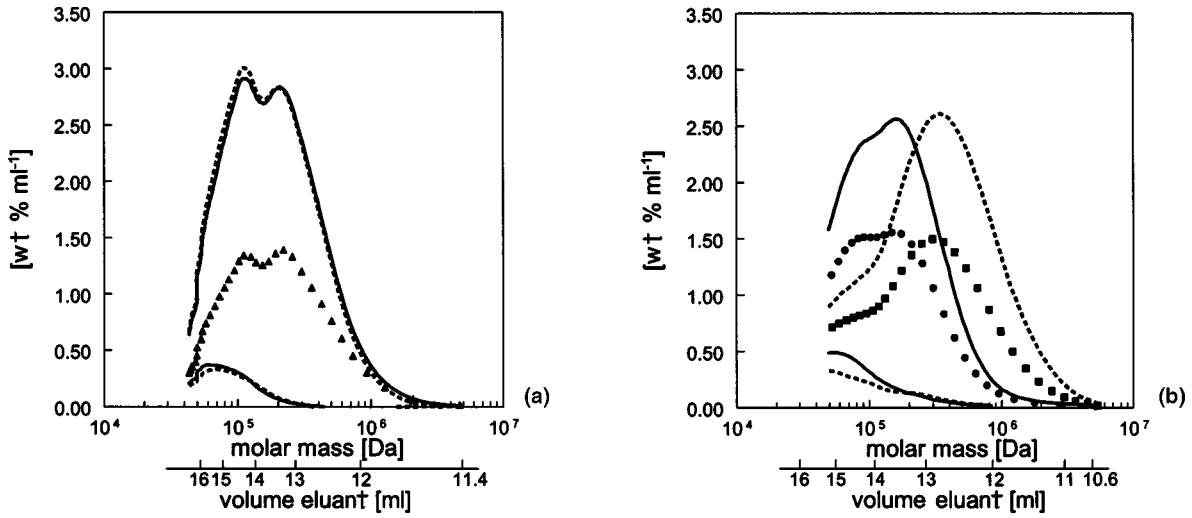

FIG. 4. The effect of dextran molar mass on the molar mass distributions in coexisting phases at 60 °C. (a) gelatin, (b) dextran. Lines upper set, rich phase; lines lower set, poor phase; symbols, components before phase separation. Mixtures: --, sample *A*2; —, sample *D*. Native material: ▲, gelatin 170 kDa; ●, dextran 148 kDa; ■, dextran 282 kDa. Overall concentration of both gelatin and dextran is 5 wt %.

Table III also gives the results for mixture *F*. From Table II it is clear that the mixture of two gelatins that is used in this sample has lower molar mass values than gelatin 170 kDa, which is used in all other samples. Figure 5 shows the molar mass distributions of samples *F* (mixture of gelatin) and *A*2 (gelatin 170 kDa) before and after phase separation. For gelatin, it turns out that decreasing the molar mass of the native material results in a decrease of the average molar mass in both coexisting phases. Together with this decrease in average molar mass values, the peaks of the distributions in both phases shift to lower molar mass values.

Comparing the molar mass distribution of dextran from sample *F* with that of sample *A*2 shows that the distribution in the dextran-rich phase is not affected by the molar mass change of gelatin. The distribution in the dextran-poor phase, however, is wider. The same conclusions can be drawn from the data in Table III(b). In the dextran-rich phase, hardly any changes occur to the dextran molar masses, while in the dextran-poor phase the molar mass of dextran increases with decreasing gelatin molar mass. It is remarkable that the final concentration of dextran in its rich phase decreases and in its poor phase increases compared to that in sample *A*2 in which only dextran 170 kDa is used.

Summarizing the effects of the molar mass of the two components, it appears that in coexisting phases gelatin, like dextran, does not affect the molar mass distribution of the other component, nor its average values of the molar mass in the enriched phase of this other component. On the other hand, the molar mass of the opposite component does affect the molar mass in the depleted phase. By increasing the molar mass of component *A*, the molar mass of component *B* decreases in its depleted phase. The influence of varying the

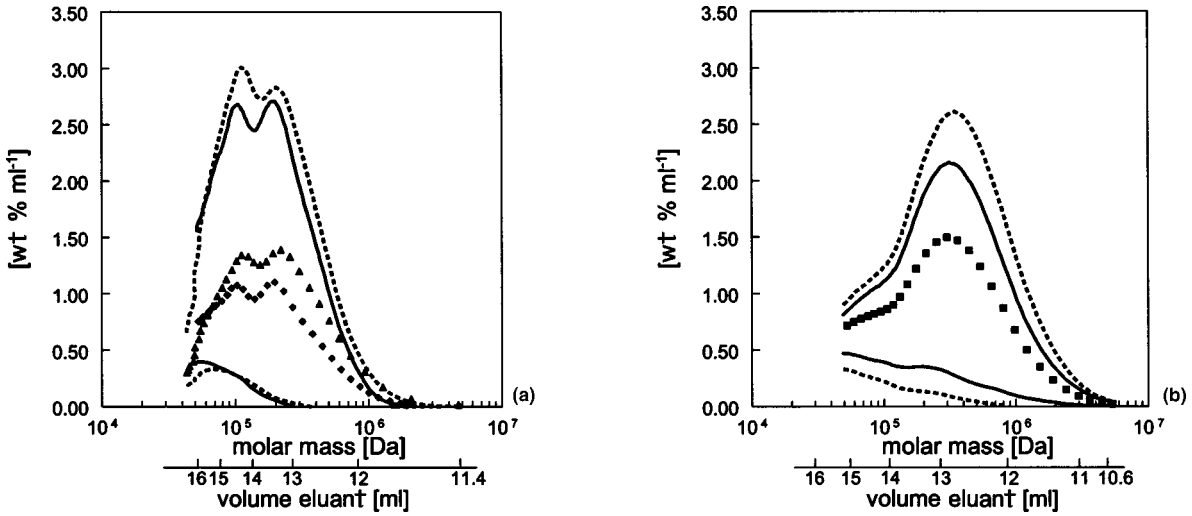

FIG. 5. The effect of gelatin molar mass on the molar mass distributions in coexisting phases at 60 °C. (a) gelatin, (b) dextran. Lines upper set, rich phase; lines lower set, poor phase; symbols, components before phase separation. Mixtures: --, sample *A*2; —, sample *F*. Native material: ▲, gelatin 170 kDa; ♦, mixture gelatin 43 kDa and gelatin 74 kDa; ■, dextran 282 kDa.

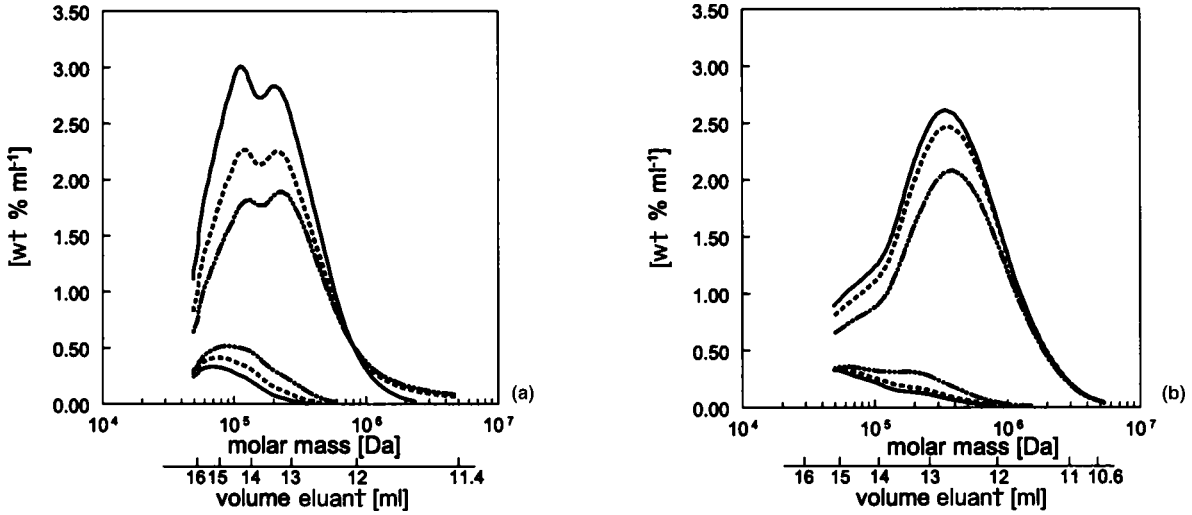

FIG. 6. Molar mass distributions in coexisting phases at 60 °C, for various total polymer concentrations. (a) Gelatin, (b) dextran. Upper set of lines, rich phase; lower set of lines, poor phase. —, sample *A*2; --, sample *B*; —.., sample *C*

molar mass of a component on its own distribution is strongest in its own enriched phase.

2. Concentration effects

The phase diagram in Fig. 2 shows the effect of diluting the system on the phase composition. It shows that, if the total initial polymer concentration decreases, the composition of the phases become more similar. This is reflected in Fig. 6. The area under the curves of gelatin and dextran in their rich phases decreases when the initial concentration polymer decreases. On the other hand, the area under the curves of gelatin and dextran in their poor phases increases on decreasing the initial concentration.

The molar mass distributions of gelatin as well as dextran in their enriched phases hardly change as function of the concentration (see Fig. 6). In contrast, the peak values of the polymers in their depleted phases increase on decreasing the initial concentration. This shift in peak values results in an increase of the values for M_n, M_w, and M_z of all components in all phases. The small increase of the molar mass values of the components in their rich phases is also due to the shift in peak value in the poor phases: the more material with a low molar mass moves to the poor phase, the higher the average molar mass in the rich phase will be.

3. Temperature effects

The results of the fractionation on the molar mass distribution as function of the temperature is given in Fig. 7. For gelatin [Fig. 7(a)] in the rich as well as in the poor phase a decrease of the molar mass with increasing temperature is observed. This effect is the strongest for the sample at 70 °C. This decrease in molar mass is probably due to temperature-induced degradation of the gelatin which is a result of the method used. From the literature [11] is known that gelatin shows this degradation, and before the samples were taken the tubes were in a water bath for 20 h.

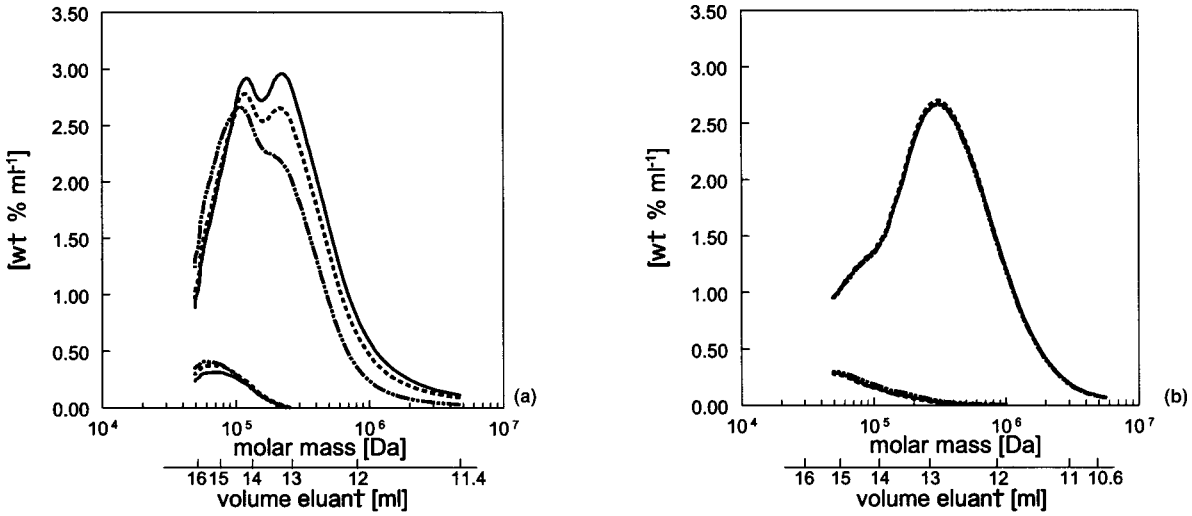

FIG. 7. The effect of temperature on the molar mass distributions in coexisting phases. (a) Gelatin, (b) dextran. Upper set of lines, rich phase; lower set of lines, poor phase. —, sample *A*1; --, sample *A*2; —.., sample *A*3.

TABLE III. M_n, M_w, and M_z and the polydispersity for (a) gelatin and (b) dextran in the different phases for the different mixtures (concentrations in wt %, molar mass averages in kDa).

(a) Gelatin

	Gelatin in gelatin-rich phase					Gelatin in dextran-rich phase				
Sample	Conc. gelatin	M_n	M_w	M_z	$\frac{M_w}{M_n}$	Conc. gelatin	M_n	M_w	M_z	$\frac{M_w}{M_n}$
*A*1	10.2	105	201	534	1.9	1.0	62	72	86	1.2
*A*2	10.2	97	175	410	1.8	1.2	62	74	92	1.2
*A*3	9.7	87	150	359	1.7	1.4	59	67	79	1.1
B	8.2	98	183	472	1.9	1.4	68	86	119	1.3
C	6.7	102	200	549	2.0	1.8	75	102	154	1.4
D	10.4	96	169	353	1.8	1.3	62	73	94	1.2
E	10.2	97	180	265	1.9	1.2	61	71	86	1.2
F	10.8	76	145	304	1.9	1.8	46	57	76	1.2

(b) Dextran

	Dextran in gelatin-rich phase					Dextran in dextran-rich phase				
	Conc. dextran	M_n	M_w	M_z		Conc. dextran	$M_n\frac{M_w}{M_n}$	M_w	M_z	$\frac{M_w}{M_n}$
*A*1	1.2	25	49	109	1.9	7.9	73	332	1020	4.5
*A*2	1.2	28	58	135	2.1	7.8	73	348	1050	4.8
*A*3	1.3	26	56	150	2.1	7.9	73	328	1000	4.5
B	1.4	29	80	231	2.8	8.0	75	382	1120	5.1
C	1.6	33	107	312	3.3	7.9	76	400	1160	5.3
D	1.4	52	130	416	2.5	7.5	62	73	94	1.2
E	1.4	29	65	157	2.3	7.7	96	250	821	3.6
F	2.2	31	128	542	4.1	6.5	65	330	1050	5.1

For dextran in its rich phase the temperature has no influence on the molar mass distribution and subsequently on the values of the molar mass. In the gelatin-rich phase the molar mass distribution of dextran becomes broader with increasing temperature. The molar mass values also increase slightly with increasing temperature.

4. Gelatin peak shape

All figures of the molar mass distribution of gelatin show a bimodal distribution of the gelatin in its rich phase, probably corresponding to a monomer-dimer equilibrium [12,13]. Apart from an overall downward shift, which is probably due to some thermal degradation, the peak also changes its shape as a function of temperature and concentration [see Figs. 6(a) and 7(a)]. Decreasing the concentration results in a shift of the peaks to a slightly higher molar mass. It also influences the ratio of the heights of the two peaks. It appears that the lower the concentration, the higher the dimer peak compared to the monomer peak. On the other hand, the molar mass of the gelatin in its depleted phase shifts to the molar mass of the monomer peak. Apparently, the gelatin from the monomer peak in its rich phase shifts to its depleted phase with decreasing starting concentration. This results in a smaller monomer peak and a relatively higher dimer peak in the gelatin-rich phase. Increasing the temperature also increases the amount of material with a lower molar mass in the gelatin-rich phase.

C. Phase separation compared with dialysis

As a result of the phase separation, molar mass distributions become fractionated between the coexisting phases in equilibrium. To investigate the influence of the fractionation on this equilibrium, dialysis experiments were carried out. With use of a dialysis membrane, the water in the system, and not the polymers, was forced to establish the equilibrium. This approach bears on the assumption that the interface between coexisting phases formed by phase separation, can be considered as a semipermeable ''liquid'' membrane, permeable to water and small polymers. For the dialysis experiments as well as the phase separation experiments, the water concentrations in both ''phases'' were determined. Figure 8 shows the results. The initial concentrations were chosen such that the concentrations in the coexisting phases were nearly the same as the concentrations inside and outside the dialysis tube. This figure shows, considering the experimental error of 10% in the determination of the concentration gelatin and dextran, that the suppression of fractionation does not significantly affect the distribution of water over the two phases, neither does temperature.

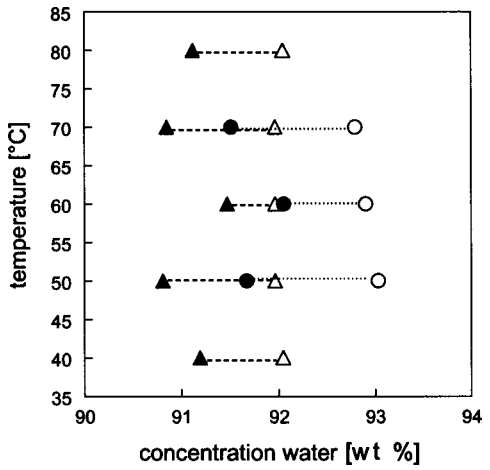

FIG. 8. Concentration of water after dialysis and phase separation in gelatin-rich (closed symbols) and dextran-rich (open symbols) phases. ▲, △, dialysis performed with 8 wt % gelatin against 8 wt % dextran. ●, ○, phase separation performed with 4 wt % gelatin and 4 wt % dextran (for final polymer concentrations, see Fig. 2). Dotted lines connect final concentrations in coexisting phases.

D. Mechanical properties of gelatin after phase separation

Figure 9 shows the results of the rheological measurements with the native gelatin and the gelatin of both phases. These results show that the gelatin from the enriched phase is able to form a stronger gel than the gelatin from the depleted phase. The reason that the native gelatin forms a stronger gel than the gelatin from both phases is probably due to the fractionation. This fractionation can be both fractionation in molar mass as well as a chemical fractionation.

IV. DISCUSSION

A. General

The study presented in this paper focuses on the effect on molar mass distribution as a result of phase separation of gelatin and dextran in aqueous solution. The general picture that emerges is that as a result of the phase separation both components of the biopolymer mixture get fractionated with respect to their molar mass. The enriched phases of both components contain preferentially the particles from the high molecular mass part of the distribution, while the depleted phases mainly contain the small particles from the distribution. It appears that the fractionation is the strongest for dextran. As a result of this fractionation it is expected that the (functional) properties of both polymers in both phases change, relative to their native material. As an example, the gel properties of gelatin taken from each of the phases were compared. It turns out that the gelatin fractions have significantly different gel strengths. This result of the fractionation should be taken into account in understanding the gel strength of gelled biopolymer based water in water emulsions [10,14].

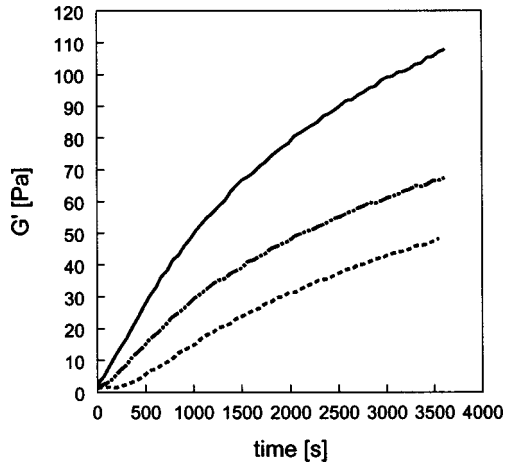

FIG. 9. G' of the gels made from gelatin after ''quench'' to 15 °C. —, native gelatin; —.., gelatin from the enriched phase; --, gelatin from the depleted phase.

The temperature has no influence on the composition of the two coexisting phases. This suggests that the phase separation is induced by excluded volume effects, as discussed in a previous paper [12]. A model for the entropically driven phase separation of small protein particles in a semidilute polysaccharide solution is given by Wang *et al.* [15]. This model could be successfully applied to the system gelatin/dextran [16]. Although one condition for the applicability of the model only exists for a small part of the distribution, i.e., a large size difference between gelatin and dextran chains, the experiments seem to be in good agreement with this model [12].

It appears that the molar mass does not affect the concentrations of gelatin and dextran in the coexisting phases. Forciniti *et al.* observed for the system polyethylene glycol/dextran that with increasing molar mass the effect of increasing the molar mass on the concentrations in the coexisting phases disappeared [17]. Apparently, the molar masses we used in this study were sufficiently large that we did not find an effect on the concentrations in the coexisting phases. However, the overall polymer concentration does affect the molar mass in the coexisting phases. Decreasing the concentration results in an increase of molar mass of gelatin as well as dextran in both phases. This is probably due to the fact that a decrease of concentration results in a decrease of excluded volume, which enables larger molecules to be present in the depleted phase. This concentration-dependent molar mass distribution was also determined by Croguennoc *et al.* [4] for the system κ-carrageenan/β-lactoglobulin/water. They observe that, with increasing κ-carrageenan concentration in the system, there is a decrease in the smallest size of the β-lactoglobulin aggregates that phase separate.

Comparing the results of the dialysis experiments and the phase separation experiments, the following conclusion can be drawn. Figure 8 shows that, whether the coexisting phases are pure solutions of gelatin and dextran separated by a dialysis membrane, or the coexisting phases are the result of phase separation without a dialysis membrane, the water concentration in the coexisting phases is the same for both cases. The ''contamination'' of the two phases with the opposite component (as is the case for phase separation without a membrane) turns out not to affect the water distribution. If

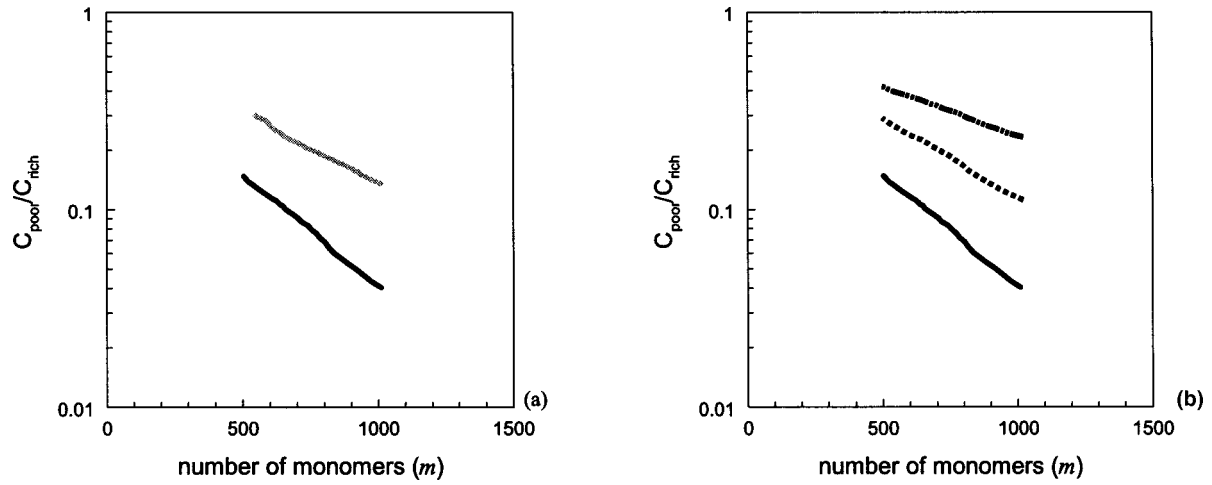

FIG. 10. $c_{x,\mathrm{poor},m}/c_{x,\mathrm{rich},m}$ as function of the number of monomers in a polymer. (a) For sample $A2$ (5 wt % gelatin 170 kDa + 5 wt % dextran 282 kDa, $T_{\mathrm{PS}}=60\,^{\circ}\mathrm{C}$): gray line, gelatin; black line, dextran. (b) Effect of concentration for dextran at $T_{\mathrm{PS}}=60\,^{\circ}\mathrm{C}$: —, sample $A2$ (5%/5%); --, sample B (4.5%/4.5%); —.., sample C (4%/4%) (all percentages w/w).

there were a strong difference in water affinity between the two polymers, one would expect a difference in water distribution between the membrane and nonmembrane cases. Such a difference is not found. Therefore, we conclude that differences in water affinity are not the main driving force for the phase separation.

B. Quantitative interpretation

In order to quantify the degree of fractionation, we introduce the quantity $c_{x,\mathrm{poor},m}/c_{x,\mathrm{rich},m}$, in which $c_{x,\mathrm{poor},m}$ and $c_{x,\mathrm{rich},m}$ are the concentrations of component x (gelatin or dextran) with a degree of polymerization m in the depleted (''poor'') and the enriched (''rich'') phase (see Fig. 10 for an example). The value of $m=1$ corresponds to a monomer. For gelatin, the monomer mass is taken to be 90 Da (the average mass of an amino acid in the gelatin used) and for dextran 162 Da (the mass of a glucose repeating unit). Figure 10 was derived by dividing, for each molar mass of the distribution, the polymer concentration in the depleted phase by the concentration of polymer in the enriched phase and plotting this value against the corresponding mass of the polymer in number of monomers.

We find that fractionation of gelatin and dextran takes place for all molar masses of the distribution. For molar masses up to approximately 1000 monomers, the fractionation is found to depend exponentially on the degree of polymerization m for each species x according to

$$\frac{c_{x,\mathrm{poor},m}}{c_{x,\mathrm{rich},m}}=\frac{V_{x,\mathrm{rich}}n_{x,\mathrm{poor},m}}{V_{x,\mathrm{poor}}n_{x,\mathrm{rich},m}}\approx C_x e^{-Am}, \tag{6}$$

where C_x is a constant, $V_{x,\mathrm{rich}}$ and $V_{x,\mathrm{poor}}$ are the volumes of the two coexisting phases, $n_{x,\mathrm{rich},m}$ and $n_{x,\mathrm{poor},m}$ are the number of particles of species x with m monomers in the rich and depleted phases, respectively, and A is a constant that depends only on the initial polymer concentration. Above 1000 monomers per chain, no reliable determinations were possible. Figure 10(a) shows that the slope of the dextran curve is steeper than the slope of the curve of gelatin. This implies a stronger fractionation for dextran than for gelatin. This is in good agreement with Figs. 4–7 and Tables III(a) and III(b). These figures and tables also suggest that the fractionation is stronger for dextran than for gelatin: the peaks of the distributions and the average molar mass values of dextran in the two phases differ more than those of gelatin. With respect to the effect of concentration, Fig. 10(b) shows (for dextran) that the lower the initial concentration, the less steep is the slope of the curve. The same can be seen for gelatin (figure not shown). This implies that the fractionation is less strong if the concentration decreases. We also observed that the degree of fractionation shows no temperature dependence (not shown).

The exponential dependence of the fractionation on molar mass, according to Eq. (6), was put forward in the literature decades ago [18–21]. In general the fractionation can be described by a Boltzmann factor

$$\frac{c_{x,\mathrm{poor},m}}{c_{x,\mathrm{rich},m}}=e^{-\Delta G/RT}, \tag{7}$$

where ΔG denotes the free energy increase involved in transferring one mole of polymer (with m monomers) from the enriched to the depleted phase. Bawn [19] correctly derived Eq. (7) on the basis of a free energy containing a heat term and an entropy of mixing term, while Brönsted erroneously ignored the latter, though arriving at the same result [20,21].

Brönsted pointed out that for large, spherical molecules, m should be replaced by the surface area of the molecule [20]. Indeed, Albertsson derived an expression for the partitioning as a function of the particle surface area and the surface tensions, i.e., between the particle and the two separated phases [18]. Assuming Antonoff's rule [22], one may write the result of Albertsson as

$$\frac{c_{x,\mathrm{poor},m}}{c_{x,\mathrm{rich},m}}=e^{-\gamma A_{\mathrm{surface}}/RT}, \tag{8}$$

TABLE IV. Overview of the values for A, $\ln_{10}(C/\rho)$ (with $\rho = V_{x,\mathrm{rich}}/V_{x,\mathrm{poor}}$), and ΔG for gelatin and dextran as functions of the initial concentration and the temperature. The value of ΔG is per mole of polymers with a length of 1000 monomers.

		Gelatin			Dextran		
T_{PS}[a] (°C)	Initial concentration (sample) (wt %)	A	$\ln_{10}\frac{C}{\rho}$	ΔG (J mol^{-1})	A	$\ln_{10}\frac{C}{\rho}$	ΔG (J mol^{-1})
50	5.0+5.0 (*A*1)	0.0018	−0.57	6.36×10^3	0.0031	−0.53	9.75×10^3
60	5.0+5.0 (*A*2)	0.0018	−0.57	6.56×10^3	0.0031	−0.53	10.05×10^3
60	4.5+4.5 (*B*)	0.0010	−0.32	3.65×10^3	0.0019	−0.53	6.73×10^3
60	4.0+4.0 (*C*)	0.0007	−0.12	2.27×10^3	0.0013	−0.52	4.85×10^3

[a]T_{PS} is the temperature at which phase separation was made to take place.

where γ denotes the interfacial tension between the two separated phases and A_{surface} the surface area of one mole of molecules with m monomers.

Table IV shows the values of ΔG for the transfer of one mole of polymers consisting of 1000 monomers from the enriched to the depleted phase at various temperatures and initial polymer concentrations. The value of 1000 monomers is chosen as a typical length. Table IV shows that with decreasing concentration the value of ΔG decreases. Assuming only entropy contributions, this would imply less difference in entropy for a polymer upon its transfer from the enriched to the poor phase. This is certainly in line with the expectation that upon decrease of initial concentration each individual polymer will indeed have access to more free volume in both phases and therefore will experience a smaller difference in entropy upon transfer from the one phase to the other.

One may equate $\gamma A_{\mathrm{surface}}$ to $\gamma m A_{\mathrm{monomer}}$, where A_{monomer} denotes the surface area of one mole of monomers. Using a typical value of 2×10^{-18} m$^2\times N_A$ for A_{monomer} [23], with N_A Avogadro's number and $m=1000$ and using the order of magnitude of ΔG in Table IV, one obtains a value for γ of 4 μN m^{-1}, which is an eminently reasonable value, considering the experimental values obtained in the range of 1 to 10 μN m^{-1} for similar systems [24].

Recently, the exponential behavior of the fractionation was also observed in computer simulations [25]. The results of these simulations and the comparison with the experimental data will be the subject of a following paper.

With use of the values for A, C_x, and ρ_x (with $\rho_x = V_{x,\mathrm{rich}}/V_{x,\mathrm{poor}}$), we can derive an expression for the number average molar mass of each polymer in each phase separately. Combining the equation

$$n_{x,m} = n_{x,\mathrm{rich},m} + n_{x,\mathrm{poor},m}, \tag{9}$$

in which $n_{x,m}$ is the total number of particles of species x with m monomers in the native material, with Eq. (6), we get

$$n_{x,\mathrm{rich},m} = \frac{\rho_x n_{x,m}}{\rho_x + C_x e^{-Am}},$$

$$n_{x,\mathrm{poor},m} = \frac{n_{x,m} C_x e^{-Am}}{\rho_x + C_x e^{-Am}}. \tag{10}$$

Assuming that Eq. (6) is valid for all molar masses present in the system, we get from Eq. (10)

$$\frac{M_{x,n,\mathrm{rich}}}{M_{\mathrm{mono},x}} = \frac{\sum_m n_{x,\mathrm{rich},m} m}{\sum_m n_{x,\mathrm{rich},m}} = \frac{\sum_m [n_{x,m}/(\rho_x + C_x e^{-Am})]}{\sum_m [n_{x,m}/(\rho_x + C_x e^{-Am})]},$$

$$\frac{M_{x,n,\mathrm{poor}}}{M_{\mathrm{mono},x}} = \frac{\sum_m n_{x,\mathrm{poor},m} m}{\sum_m n_{x,\mathrm{poor},m}} = \frac{\sum_m [n_{x,m} m C_x e^{-Am}/(\rho_x + C_x e^{-Am})]}{\sum_m [n_{x,m} C_x e^{-Am}/(\rho_x + C_x e^{-Am})]}, \tag{11}$$

with $M_{\mathrm{mono},x}$ the monomer mass of polymer x. If the function of the molar mass distribution of the native material $[P(m)]$ is known, Eq. (11) can then be written as

$$\frac{M_{x,n,\mathrm{rich}}}{M_{\mathrm{mono},x}} = \frac{\int_0^\infty [P(m) m/(\rho_x + C_x e^{-Am})]\,dm}{\int_0^\infty [P(m)/(\rho_x + C_x e^{-Am})]\,dm},$$

$$\frac{M_{x,n,\mathrm{poor}}}{M_{\mathrm{mono},x}} = \frac{\int_0^\infty [P(m) m C_x e^{-Am}/(\rho_x + C_x e^{-Am})]\,dm}{\int_0^\infty [P(m) C_x e^{-Am}/(\rho_x + C_x e^{-Am})]\,dm}. \tag{12}$$

C. Practical consequences

The fractionation in molar mass also affects the mechanical properties of the gel that can be made from the gelatin from both phases. From the measurements of the storage modulus it turns out that the gelation properties of the gelatin are affected by the fractionation. As we expected, the gelatin

from the poor phase is not able to form a gel that is as firm as the gel that can be made from the gelatin from the rich phase. Ferry and Eldridge already showed that the higher the molar mass of the gelatin, the higher the gel strength of the gel it forms [11,26]. The differences in the gel forming properties may be caused by either the amount of helices that can be formed or the length of the junction zones that are formed. Apart from physical fractionation (fractionation in molar mass), chemical fractionation can take place during phase separation. Due to this chemical fractionation, the gelatin in the two coexisting phases has a different chemical composition and, consequently, different gel forming properties. Surprisingly, the gel of the native gelatin forms a firmer gel than the gelatin in the two coexisting phases. This may be caused by chemical fractionation.

The gelatin graphs in Fig. 4–7 show a double peak for the gelatin in the gelatin-rich phase. From other studies [13] it is known that gelatin has a temperature-dependent molar mass distribution even above the gelation temperature of gelatin (~30 °C). Figure 4 shows that the shape of this bimodal distribution is affected by the fractionation. The fractionation apparently affects the monomer-dimer equilibrium of the gelatin. This change in the distribution of the gelatin-rich phase compared to the distribution of the pure gelatin may be related to the fact that the pure gelatin is able to form a firmer gel than the gelatin from the gelatin-rich phase.

The differences in gelation properties between the gelatin in the poor and rich phases were not taken into account in earlier work [6–10]. However, the present work shows that ignoring differences in the gelling properties of material in coexisting phases might lead to erroneous results for the calculation of the strength of mixed (phase separated) gels.

V. CONCLUSIONS

The phase separation of aqueous mixtures of gelatin and dextran results in a strong fractionation in molar mass of the two polymers. It appears that the overall concentration is the only factor that influences the molar mass distribution of the polymers in each phase. The temperature does not affect the distribution. For the lower molar mass part of the distribution (up to 1000 monomers), the fractionation is found to be exponential in the molar mass of the polymer. Interpreting this exponential ratio as a Boltzmann factor, the free energy involved in transferring a polymer with a certain length from the enriched to the depleted phase can be calculated. Using reasonable values for molecular dimensions we arrive at a reasonable value for the interfacial tension between the two phases.

It appears that the low molar mass part of the distribution has the same influence on the osmotic pressure whether it is in the depleted phase of a system in which species can move freely, or in the enriched phase of a forcibly separated system (using a dialysis membrane). This implies that water affinity is no driving force for the phase separation. In addition it is concluded that fractionation does not influence the total polymer concentration in the two coexisting phases. As a result of the fractionation, the gelling properties of the gelatin in coexisting phases change as compared to the properties of the native gelatin. This implies that the fractionation has to be taken into account when calculating the mechanical properties of a mixed phase separated gel containing gelatin.

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