Partitioning of Anionic/Nonionic Polysaccharides in a Segregative System of Anionic Polyelectrolyte Mixtures in Aqueous Solution: Effects of Polymer Charge Density and Ionic Strength of Solution

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ABSTRACT: The partitioning of three polysaccharides, Dextran sulfate (DxS), λ -Carrageenan (CAR), and Dextran (Dx), and its dependence on polymer charge density and ionic strength have been investigated in the segregative mixture of poly(sodium styrene sulfonate) (NaPSS) and sodium polyacrylate-acrylamide copolymers, NaP(AA/AM), of variable charge density, in aqueous solutions. It is shown that the solubility of all the three may be zero or finite in one or both phases, but the preferred phase is determined by the charge density on the copolymer. When charge densities are equal on the phase forming polymers, the saccharides CAR and Dx favor the phase rich in PSS, but they have greater

INTRODUCTION

Phase separation is a common feature of mixed polymer solutions.¹⁻⁴ Various applications exploit the tendency towards phase separation in polymer mixtures. One such application is the aqueous two-phase partitioning of biological macromolecules,^{1,5} such as partitioning of cells and purification of proteins.^{6–9} However, the aqueous two phase systems employed are often formed by polymer-surfactant mixtures, mixed micelles, or micellar two phase systems consisting of only one surfactant.^{7–13}

Despite the considerable amount of research in the field of segregating polymer mixtures, the molecular interactions in the systems are inadequately understood, but theoretical models have been proposed.^{2,14–22} The better the interactions that govern polymer mixing in water are understood, the better one is able to control practical separation processes. The majority of systems studied previously have consisted of mixtures of nonionic macromolecules.⁵ There have, as of yet, been comparatively few studies on

affinity for the polymer of lower charge density when the charge densities on phase polymers are unequal. The partitioning behavior of DxS is the opposite of the other two polysachharides with respect to which phase it has the greatest affinity for. The values of the partitioning coefficients show that the system may be potentially useful for the purification of polysaccharides and other macromolecules, if appropriate conditions are selected. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 1728–1734, 2006

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partitioning in mixtures of similarly charged polyelectrolytes.^{23–25} Such systems may have advantages over uncharged systems in the separation of proteins because of the tuneable charge in the system, arising from the dissociated counterions of the polyelectrolytes.^{25,26}

In this study, the two phase system employed for partitioning is made up of two segregating polyanions, poly(styrene sulfonate) sodium salt (NaPSS or PSS), and polyacrylate sodium salt (NaPAA or PAA). The charge density of the latter has been varied by substituting it with poly(acrylate-*co*-acrylamide) (PAM) with 20% carboxylation on the polymer chain. The phase behavior of this system was subject to investigation in a previous study by the same authors.²⁴ This study is devoted to the partitioning of a third component, with similar or no charge, in the system. Three such components are investigated. They are the anionic polysaccharides λ -Carrageenan (CAR) and Dextran Sulfate (DxS), and the uncharged polysaccharide Dextran (Dx).

EXPERIMENTAL

Materials

Journal of Applied Polymer Science, Vol. 100, 1728–1734 (2006) © 2006 Wiley Periodicals, Inc. Polyacrylic acid sodium salt of molecular weight 225 kDa (PAA225) was purchased from Polysciences, as a

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solution of 20 wt % of solids in water, and used without further purification. Polystyrene sulfonate sodium salt of molecular weight 500 kDa (PSS500) was obtained from Scientific Polymer Products, Inc. The latter contained some insoluble materials that were removed by centrifugation. The pH was adjusted with NaOH (s) to a high value, to ensure complete dissociation of the carboxyl groups on the PAA chain in solution.²⁷ This ensured that the fraction of charged monomers on the polyacid was maintained at or near unity. PSS is a strong electrolyte and will be fully dissociated even at low pH.

Poly(acrylamide-co-acrylic acid) copolymer (PAM-τ, where τ denotes degree of hydrolysis) had molecular weight 200 kDa and was purchased from Aldrich (τ equal to 20%). Potentiometric titration²⁸ of this component showed the degree of hydrolysis to be equal to the nominal value (20%). Because the pH was adjusted with addition of NaOH to ensure full ionization of the acrylate units in solution, there is a possibility of hydrolysis of acrylamide groups on the polymer backbone during equilibration of samples. To determine the extent of autohydrolysis a polymer solution was prepared for segregating samples, but without the PSS component. Aliquots were taken and analyzed on consecutive days to see if the degree of hydrolysis increased. It was found that it was increased by about 32% the first day and about 33% after four days. The component with a nominal value of 20% hydrolysis on the polymer chain was therefore in reality closer to 30% hydrolyzed, when NaOH was present, but will be referred to as PAM20. λ -Carrageenan (CAR), high MW, was obtained from Sigma. Dextran (Dx, MW: 500 kDa, Leuconostoc mesenteroides) was obtained from Sigma and Dextran Sulfate (DxS, MW: 500 kDa, Leuconostoc ssp.) was obtained from Fluka.

Carrageenan is generic term for a group of galactan sulfates extracted from red seaweed. Their backbone is formed of $(1\rightarrow3)$ -linked D-galactopyranose residues alternating with $(1\rightarrow4)$ -linked D-galactopyranose residues that carry various sulfated groups. The three principal types are *Kappa*, *Iota*, and *Lambda*. *Kappa* and *Iota* are gelling polymers, but *Lambda* is a thickening polymer. Dextran is a 1–6-linked α -D-glucopyranosyl backbone polysaccharide and its sulfated form, DxS, is a polymer of α -1,6-linked glucopyranose units with sulfate groups substituted for hydroxyl groups. The 1 \rightarrow 6 linkages give the chain a very high flexibility.

Phase compositions

Segregating samples containing polysaccharide for partitioning studies were prepared and stored at 25 $(\pm 1)^{\circ}$ C. Equal amounts of each phase forming polymer were present in all prepared samples. Compositions of all samples as prepared are given in Table I. When phase separation was achieved, the samples

 TABLE I

 Experimental Design for the Partitioning Experiments

	Levels of variables						
Samples	Solvent	Ionic strength	NaOH	CAR/ DxS/Dx	Charge density		
Exp 1	-1	-1	-1	-1	-1		
Exp 2	1	-1	-1	-1	-1		
Exp 3	-1	1	-1	-1	-1		
Exp 4	1	1	-1	-1	-1		
Exp 5* [§]	-1	-1	1	-1	-1		
Exp 6*	1	-1	1	-1	-1		
Exp 7* [§]	-1	1	1	-1	-1		
Exp 8*	1	1	1	-1	-1		
Exp 9	-1	-1	-1	1	-1		
Exp 10	1	-1	-1	1	-1		
Exp 11	-1	1	-1	1	-1		
Exp 12	1	1	-1	1	-1		
Exp 13	-1	-1	1	1	-1		
Exp 14	1	-1	1	1	-1		
Exp 15	-1	1	1	1	-1		
Exp 16	1	1	1	1	-1		
Exp 17	-1	-1	-1	-1	1		
Exp 18	1	-1	-1	-1	1		
Exp 19	-1	1	-1	-1	1		
Exp 20	1	1	-1	-1	1		
Exp 21* [§]	-1	-1	1	-1	1		
Exp 22*	1	-1	1	-1	1		
Exp 23* [§]	-1	1	1	-1	1		
Exp 24*	1	1	1	-1	1		
Exp 25	-1	-1	-1	1	1		
Exp 26	1	-1	-1	1	1		
Exp 27	-1	1	-1	1	1		
Exp 28	1	1	-1	1	1		
Exp 29	-1	-1	1	1	1		
Exp 30	1	-1	1	1	1		
Exp 31	-1	1	1	1	1		
Exp 32	1	1	1	1	1		
33 centre point	0	0	0	0	0		
34 centre point	0	0	0	0	0		
35 centre point	0	0	0	0	0		

All experiments were carried out for partitioning of CAR. Eight experiments were selected for partitioning of DxS,. These are denoted with an asterisk in the table. Four experiments were conducted for partitioning of Dx. These are marked with § in the table. The numbers -1, 0, and 1 denote high, moderate, or low levels for the variables [Level -1: solvent -80%, ionic strength -0M NaC1; NaOH -0%; CAR/DxS -0.1%; charge density -20%; Level 0: solvent -85%, ionic strength -0.5M NaC1; NaOH -0.04%; CAR/DxS -0.3%; charge density -60%; Level 1: solvent -90%, ionic strength -1M NaC1; NaOH -0.61%; CAR/DxS -0.5%; charge density -100%]. Each experiment is a unique combination of the five variables.

were centrifuged at 2600 rpm for 1–3 h. After centrifugation, the separated phases were collected separately and analyzed. The PSS concentration in the samples was determined by UV absorption at 249 and 261 nm and was calculated from double calibration to minimize the influence of the PAA or PAM20 component. The analytical value was within an interval (± 0.5 wt %) around the real value estimated from analyses on test solutions. The concentration of PAA or PAM20 was determined by refractive index measurements, taking into account the contributions from the previously determined PSS concentration and the salt and NaOH concentrations as prepared. The contribution from each of the four constituents to the refractive index were determined by multiple linear regression on a set of calibration samples. The experimental error for PAM20 determination was mostly influenced by the uncertainty in PSS and NaOH concentrations and found to lie within the interval ± 0.5 wt % around the real value. For our purpose, this is an acceptable degree of accuracy, as the main concern is the correct identification of the PSS and P(AA/AM) rich phases.

Partitioning

The segregating samples containing polysaccharide prepared as described above were used. To simultaneously determine the effects of polymer concentration, ionic strength, charge density, pH and polysaccharide concentration, experimental design²⁹ was employed. A full factorial design with five variables that are varied on two levels gives a total of 32 samples in the series of experiments for partitioning of CAR (Table I). The main effect of each variable may be calculated as the difference between the average response value at high and low levels of the variable. In addition, synergistic effects of combined variables can be quantified by using experimental design. Unfortunately, the full potential of such designs could not be exploited because of the limited solubility of CAR, and so the results were interpreted in a largely qualitative manner instead.

For DxS partitioning, eight experiments were carried out, where polymer concentration, ionic strength and charge density were varied. Four experiments were carried out for Dx partitioning, in which ionic strength and charge density were varied. The four experiments involving uncharged Dx were conducted at high polymer concentration, since the large viscosity difference between the phases facilitated separation of the phases before analyses. Table I contains 35 experiments. The last three are replicated center points, in which all the variables take on values intermediate between the high and low levels.

The phase compositions of segregating polymers were determined as described above and the phase concentrations of the polysaccharide was determined by the phenol-sulfuric acid method³⁰ which involves reacting the saccharide present with phenol and sulfuric acid followed by UV spectroscopy. The wavelength for maximum absorbance was 490 nm for pure saccharide solutions, and the method was found to be very accurate. However, because of the presence of PSS in the sample, which produced a turbid precipitate in the reaction, the full spectra were collected for each phase aliquot. A section of the spectra between

approximately 450 and 555 nm were analyzed by PLS regression. The spectra were differentiated before regression analysis to remove the baseline contribution from the turbidity caused by PSS in the sample. On the basis of a calibration set that spanned the appropriate concentration ranges of both PSS and polysaccharide, the concentrations of polysaccharides were determined from the differentiated spectra of the phase aliquots after reaction with phenol and sulfuric acid.

This method is very sensitive to the presence of saccharide. So even before UV analyses it is possible to estimate whether the phase aliquot contained zero, some, or a high amount of saccharide by the intensity of the color resulting from the reaction.

The partitioning coefficient is calculated as the ratio of the concentration of polysaccharide in the PAA rich phase (C_{pax}) to the concentration in the PSS rich phase (C_{pss}). The accuracy and precision of the analyses were estimated from triplicate analyses of samples of known compositions. The standard deviations were in the region of 0.05% (w/w) for the phase polymers and 0.015% (w/w) for the polysaccharide.

RESULTS AND DISCUSSION

Phase behavior

The phase compositions for the system containing CAR are shown in Table II. The partitioning coefficients of all the three polysaccharides are also shown in Table II.

The experiments on CAR partitioning involved five variables. One of these was the initial concentration of CAR. This was varied partially to see if the component had an influence on the phase behavior of the system, but also to see if the partitioning coefficient was influenced by the amount of CAR present. The phase behavior of this particular system was investigated in a previous study,²⁴ but a few observations on the phase behavior should be noted before discussing the partitioning further.

When the polysaccharide was added to the samples it was not always miscible with either phase, so it formed a separate "phase" in some samples. This is not remarkable while considering CAR is negatively charged, as were the phase polymers, and of high molecular weight.

The tie-lines of the systems containing CAR were not significantly and qualitatively different from those obtained for the same system without CAR. This was true for most of the samples.

However, there are three samples that did not segregate and two samples that exhibited altered phase behavior, presumably on account of the presence of CAR. Samples number 1, 2, and 9 did not segregate because in these samples the charge density on one of the polymers is low (PAM20), the ionic strength is

	Top phase			Bottom phase			$K(CAR)^*$	K (DxS)	$K(\mathbf{D}\mathbf{v})$
Samples	%P (AA/AM)	%PSS	%CAR	%P (AA/AM)	%PSS	%CAR	(C_{pax}/C_{pss})	$(C_{\text{pax}}/C_{\text{pss}})$	$(C_{\text{pax}}/C_{\text{pss}})$
Exp 1a	9.86	9.92	0.09						
Exp 1b	9.89	9.89	0.07						
Exp 1c	9.81	9.97	0.09						
Exp 2a	4.72	5.06	0.06						
Exp 2b	4.75	5.03	0.10						
Exp 2c	4.72	5.05	0.09						
Exp 3	21.04	0.45	0.13	0.00	28.63	0.10	1.5		
Exp 4	7.25	2.98	0.35	5.40	5.34	0.05	7		
Exp 5	15.05	2.64	0.09	6.69	13.71	0.05	2	0	20
Exp 6	5.99	2.78	0.07	0.98	10.48	0.02	3.5	0.7	
Exp 7	16.47	1.42	0.07	0.00	28.49	0.25	0.5	0.3	6
Exp 8	6.70	2.05	0.12	0.00	17.18	0.01	12	0.8	
Exp 9a	10.32	10.54	0.15						
Exp 9b	10.56	10.31	0.14						
Exp 10	4.17	5.84	0.10	7.81	2.13	0.88	9		
Exp 11	16.80	2.24	0.34	0.20	20.98	0.88	0.5		
Exp 12	7.11	2.76	0.54	2.40	10.48	0.06	9		
12 midt	4.86	6.36	0.10						
Exp 13	15.51	2.84	0.35	7.69	13.20	0.11	3		
Exp 14	6.29	2.65	0.28	0.00	11.55	0.38	1		
Exp 15	17.22	1.36	0.32	0.00	24.64	0.93	0.5		
Exp 16	6.67	1.89	0.23	0.00	17.92	0.03	8		
Exp 17	0.00	22.92	0.03	16.59	1.73	0.00	0		
Exp 18	1.69	8.73	0.00	5.43	3.57	0.00			
Exp 19	15.56	1.65	0.00	1.05	23.39	0.24	0		
Exp 20	0.40	11.57	0.04	6.50	2.68	0.01	0.2		
Exp 21	0.00	22.53	0.02	16.30	1.52	0.00	0	10	0.4
Exp 22	0.50	10.17	0.04	6.60	2.17	0.03	0.7	57	
Exp 23	15.56	1.44	0.00	0.19	24.60	0.27	0	3	0
Exp 24	0.30	11.41	0.03	7.08	2.00	0.00	0	5	
Exp 25	0.00	23.17	0.10	17.56	1.70	0.01	0.1		
Exp 26	1.19	9.97	0.06	6.19	3.08	0.02	0.3		
Exp 27	16.02	1.59	0.00	0.64	22.87	1.15	0		
Exp 28	1.06	11.87	0.04	7.85	1.82	0.00	0		
Exp 29	0.00	22.77	0.03	17.79	1.23	0.00	0		
Exp 30	0.16	11.64	0.08	7.17	2.26	0.02	0.2		
Exp 31	15.78	1.60	0.02	0.39	23.29	1.26	0		
Exp 32	0.93	11.68	0.14	7.68	1.88	0.04	0.3		
Exp 33	9.58	4.68	0.15	6.94	10.23	0.06	2.5		
Exp 34	8.99	4.92	0.11	3.71	13.60	0.03	4		
Exp 35	9.51	4.48	0.12	3.38	14.29	0.04	3		

TABLE II Phase Compositions for All Segregated Systems and Partitioning Coefficients for λ-Carrageenan (CAR), Dextran Sulphate (DxS), and Dextran (Dx)

Phase compositions are from experiments with CAR, but are similar for DxS and Dx experiments. The shading indicates experiments with high ionic strength. Experiments with odd numbers involve high polymer concentration and those with even numbers involve low(er) polymer concentration. Experiments 1–16 contain PAM20 and experiments 17–24 contain PAA.

* values to the nearest 0.5 unit (1–16) and one decimal (17–32).

low, and NaOH is not present. Without added NaOH the carboxyl groups on PAM20 are not all ionized and the observed miscibility of the phase polymers in these samples is essentially due to polyelectrolyte effects.^{17,24} Note that even at low pH, but high ionic strength (samples 3 and 4), the system segregates, so both low pH and low ionic strength are required to make samples 1, 2, and 9 homogenous. The compositions of these samples are included in Table II as an illustration of the accuracy of the analyses.

Sample 10 is anomalous with respect to phase behavior. Like sample 2, sample 10 should be homogenous because it only differs in composition from sample 2 in the higher concentration of CAR. It is possible that the higher concentration of CAR induces phase separation through its counterions, which will contribute to an increase in the ionic strength of the solvent.

Sample 12 is distinguished from all other samples by the observation that it formed three phases, of approximately equal volume. This was not observed previously.²⁴ It is given by Gibbs phase rule that a four-component mixture may form three phases at constant temperature and pressure. So it is probably more remarkable that none of the other samples ex-

					Duplicate Avg.	
Samples	Var 1 (solvent)	Var 2 (lonic strength)	Var 3 (NaOH)	Var 5 (charge density)	K (CAR)	K (DxS)
1 + 9	-1	-1	-1	-1		
2 + 10	1	-1	-1	-1	9	
3 + 11	-1	1	-1	-1	1	
4 + 12	1	1	-1	-1	8	
5 + 13	-1	-1	1	-1	2.5	0
6 + 14	1	-1	1	-1	2	0.7
7 + 15	-1	1	1	-1	0.5	0.3
8 + 16	1	1	1	-1	10	0.8
17 + 25	-1	-1	-1	1	0.05	
18 + 26	1	-1	-1	1	0.3	
19 + 27	-1	1	-1	1	0	
20 + 28	1	1	-1	1	0.12	
21 + 29	-1	-1	1	1	0	10
22 + 30	1	-1	1	1	0.5	57
23 + 31	-1	1	1	1	0.0	3
24 + 32	1	1	1	1	0.13	5

TABLE III Partitioning Coefficients (Response Variable) and Calculated Relative Effects of Variables

hibit this type of phase behavior than the fact that this sample did.

Samples 7, 11, 15, 19, 23, 27, and 31 form a concentrated, viscous, gel-like, PSS rich bottom phase in equilibrium with a less viscous top phase. This feature is not shared by any of the other samples but occurs with unfailing regularity, and the common variables are total polymer concentration (high) and ionic strength (high). Regardless of the other variables, the system arrives at equilibrium between two phases of very different physical characteristics, when those two variables take on high values. This is a feature of the system and is not due to the presence of polysaccharides.

Partitioning of car in systems of unequal charge densities

The results for this system are shown by samples 1 to 16 in Table II. If the concentration of CAR significantly influenced the partitioning, this would be evident from a comparison of partitioning coefficients in Table II for samples 1–8 with those for samples 9–16, because sample 1 and 9 are equal in all respects but the concentration of CAR, and so are 2 and 10, 3, and 11, etc. Samples 1, 2 and 9 cannot be compared.

It is readily established from this comparison that the partitioning coefficient is non-negligibly changed, from low to high CAR concentration, only between samples 6 and 14 and samples 8 and 16. These samples were of high ionic strength and low polymer concentration, and the effect of increasing the concentration of CAR is to reduce the coefficient. In other words, the concentration of CAR in each phase becomes more even when its total concentration is increased, but only at high ionic strength and low polymer concentration. Overall, the concentration of CAR did not influence partitioning significantly.

In the following discussion, the samples containing high and low CAR concentrations, but which are equal with respect to the remaining variables, will be regarded as duplicates. A new set of data can then be extracted from Table II, by taking average values of K (CAR) for these duplicates. The new set of results is presented in Table III.

The pH is likely to influence partitioning through its influence on ionization of the carboxyl groups on PAM20. At high pH the carboxyl groups are all ionized (dissociated). An increase in the partitioning coefficient upon adding NaOH would imply that CAR partitions to a greater extent into the PAM20 rich phase when all the carboxyl groups on this component are ionized. A reduction of K (CAR) upon adding NaOH would imply that when the dissociation degree of carboxyl groups on PAM20 is reduced and the polymer has a lower charge density than the nominal 20% fraction of charged units, CAR has an even higher affinity for this phase. The latter should be expected. However, from the values in Table III, it is seen that the overall effect of NaOH is negligible, which is slightly surprising. The only situation in which it has a non-negligible influence is at low ionic strength and low polymer concentration (compare row 2 with row 6 in Table III). But this result is, in fact, quite reasonable because at low ionic strength the charge density is of greater importance than it is at high ionic strength. The pH thus has the expected effect when the ionic strength is low. For high polymer concentration the comparison fails because samples 1 and 9 did not segregate.

The effect of ionic strength on partitioning is found by comparing the pairs: row 1 with row 3, row 2 with row 4, row 5 with row 7, and row 6 with row 8 in Table III. No value is available for row 1, but from the remaining values it is found that the only situation in which added salt causes a significant change in the value of K (CAR) is at low polymer concentration (high solvent content) and high pH (row 6 vs row 8). This is another manifestation of electrostatic screening effects, because at high pH the carboxyl groups are all ionized, and addition of salt reduces the repulsive interaction between PAM20 and CAR, which causes the latter to partition into the phase rich in PAM20 to an even greater extent than in the absence of salt.

Seemingly, the most important variable in the PSS-PAM20 two phase system is the polymer concentration. Quite clearly, the partitioning coefficient is higher for samples of lower total polymer concentration. This is likely to be caused by the limited miscibility of CAR with any of the phase polymers. It clearly is more miscible with PAM20 than with PSS, but as the phases become more concentrated, its solubility limit is exceeded in either phase.

Incidentally, the partitioning coefficient for the center point experiments (samples 33–35) is also above unity, so CAR still has a higher affinity for the PAM phase than the PSS phase when the former carries an intermediate charge density of 60% carboxyl groups on the polymer chain.

Partitioning of car in systems of equal charge density

The results for the system PSS-PAA are shown by samples 17–32 in Table II. The partitioning coefficient for all samples of this system is very low (below unity) or zero. Clearly, the charge density on the polymer chains has a dominant influence on the partitioning behavior of this polysaccharide. In the PSS-PAA mixtures, CAR was more miscible with the PSS rich phase, regardless of ionic strength, polymer concentration, and pH. This is the reverse of what was true for the PSS-PAM20 mixtures discussed earlier.

Comparing CAR, DxS, and Dx

The partitioning coefficients for the polysaccharides are given in Table II. Dextran displayed the same preferences as CAR, but its affinity for PAM20 is even stronger, which is seen by the comparatively high values of K (Dx) in the PSS-PAM20 system. Dextran and PAA or PAM are miscible.³¹ So it is not surprising that Dx partitions into the PAM20 rich phase. However, it prefers an environment of fully charged PSS to fully charged PAA, and so it is evidently more compatible with the former than with the latter. Hence, CAR and Dx seem to prefer the environment of the most lowly charged polymer, but change their preference to PSS over PAA when these phase polymers are equally charged.

Increased ionic strength reduced the partitioning coefficient of Dx. This was especially true in the PSS-PAM20 system, where it was reduced by approximately two thirds of its original value. The reason is most likely to be electrostatic screening of charges on PSS, which made Dx more miscible in the PSS rich phase. The conformation of PSS will be more compact in higher ionic strength solutions,³² and this may also have a role in enhancing the miscibility.

For DxS the relative affinity was the reverse of those of the other two, since K (DxS) is below unity when K (CAR) and K (Dx) are higher than unity, and *vice versa*. DxS showed a comparatively very strong affinity for the PAA rich phase in the fully charged system, but in PSS-PAM20 systems it showed greater affinity for the PSS phase.

Increasing the ionic strength had the effect of increasing the partitioning coefficients of DxS slightly in the PSS-PAM20 systems, but it dramatically reduced it in the PSS-PAA systems. This implies that whichever phase was preferred, that phase became even more favored in the presence of salt because of screening of repulsive interactions. The polymer concentration had a similar effect on DxS partitioning as it did in the case of CAR, but of greater magnitude.

CONCLUSIONS

Partitioning experiments indicated that in the aqueous segregative systems of sodium poly(styrene sulfonate) (PSS) and sodium polyacrylate (PAA), or poly(acrylate-*co*-acrylamide) (PAM20), the charge density on the phase polymers was the dominating factor in controlling partitioning behavior of the polysaccharides λ -carrageenan, Dextran sulfate, and Dextran.

In the PSS-PAM20 system, the polysaccharides λ -Carrageenan and Dextran favored the phase low in PSS, but in the PSS-PAA system they were intrinsically more miscible with the PSS component than PAA. For the polysaccharide DXS, the reverse was true. The fact that the polysaccharides have different affinities for the segregated phases implies that the system may be applied in the separation of polysachharides or other biomacromolecules.

The main objective of the study was to observe partitioning in a segregative system of two polyanions, and it is as such an exploratory investigation, and the empirical results are presented. They have been interpreted with caution, and so the conclusions are largely qualitative.

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