havior in swelling required extrapolation of the values of $w'(\lambda) - w'(1)/\lambda$, we used the Gaylord–Douglas[22] model as empirically fit to our data to objectively carry out the extrapolations. The form of $w'(\lambda)$ in this case is $w'(\lambda) = A(\lambda - 1/\lambda) + B(1 - 1/\lambda)$. As seen by the lines in Figures 3 and 4 the model fits the data very well. Furthermore, the extrapolations were not large, and we found little difference between the use of this equation and a simple graphical extrapolation by hand.

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(23) Flory, P. J.; Tatara, Y.-I. *J. Polym. Sci., Polym. Phys. Ed.* **1975**, *13*, 683.

(24) Another possibility here is that the value of $w'(1)$ in the strain energy function changes as a function of the solvent or swelling deformation. We are not pleased with this prospect and are in the process of carrying out experiments to measure the elastic properties of rubber swollen to different amounts at different vapor pressures (isopiestically) rather than in different solvents to assess this problem We prefer to think that in the undistorted configuration ($\lambda_1 = \lambda_2 = \lambda_3 = 1$) the elastic free energy function is at a minimum, which would require $w'(1) = 0$ for the isotropic (swelling) deformation.

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(26) Neuberger, N. A.; Eichinger, B. E. *Macromolecules* **1988**, *21*, 3060.

(27) Richard Gaylord of the University of Illinois considered this possibility and talked with us about it in March, 1988. It implies that the dilatational modulus[25,26] S is written as

$$S = \lambda_s \ln a_c/a_u = (\chi_c - \chi_u)\lambda_s^{-5} + \frac{V_1 w'(\lambda_s)}{RT\lambda_s}$$

where a is the activity, χ is the Flory–Huggins interaction parameter, the subscripts c and u refer to the cross-linked and un-cross-linked rubbers, respectively, and the other symbols are as defined in the text. Then, if $w'(1) = 0$ as postulated in ref 24, the fact that S is not zero at zero swelling is indicative that $\chi_c \neq \chi_u$.

(28) Arndt, K. F.; Hahn, I. *Acta Polym.* **1988**, *39*, 560.

(29) Deloche, B.; Samulski, E. T. *Macromolecules* **1988**, *21*, 3107.

(30) **Note Added in Proof:** We have recently found that simple application of eq 8 or 9 to predict the tensile response of the swollen rubber is not as successful as the predictions observed here for the compressive behavior. This is being explored in detail and will be reported upon in a future publication.

Effects on the Cloud Point of Aqueous Poly(ethylene glycol) Solutions upon Addition of Low Molecular Weight Saccharides

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ABSTRACT: Cloud point measurements are reported for aqueous poly(ethylene glycol) solutions with an added third component. The added third component is in all cases a saccharide with glucose as the monomer. The following saccharides have been investigated: glucose, maltose, cellobiose, isomaltose, maltotriose, isomaltotriose, and β-cyclodextrin. All saccharides except cyclodextrin are found to decrease the cloud point in the poly(ethylene glycol) solution. The phase diagram of a mixture of glucose with PEG20000 and water is also studied. Both the phase diagram and the cloud point curve are reasonably well described by a recently presented mean-field theory. Finally a tentative explanation is given for the differences between the saccharides in their ability to decrease the cloud point in aqueous poly(ethylene glycol) solutions.

Introduction

The use of aqueous two-phase polymer systems for purification and partitioning purposes in biochemistry has long been an established and generally used method.[1] Since systems suitable for laboratory use were developed at an early stage (usually being those with dextran and poly(ethylene glycol)), there has been relatively little work done to explain and understand the fundamental mechanisms that cause polymer incompatibility. With today's advances within biotechnology, there is great interest in finding separation methods that are suitable for large-scale applications with respect to both performance and economy. One separation method that shows great promise for large-scale applications is the use of aqueous two-phase polymer systems, if the cost problem could be solved. There is thus great interest in finding new phase systems that are both cheap and effective.[2] This work would of course be facilitated if basic physical knowledge concerning phase separations in aqueous polymer systems was available. Consequently, we have initiated a project, financed by grants from the National Swedish Board for Technical Development (STU), with the aim of studying the fundamental molecular mechanisms leading to polymer incompatibility in aqueous systems. In previous work we concluded that the basic mechanisms for incompatibility in aqueous polymer systems are well described with a modified Flory–Huggins model.[3-5] This means that the explanation of phase separation is not to be found in some specific property of the solvent but is rather to be found in the molecular interactions between the different monomer segments of the polymers. As we have pointed out earlier, a crucial point in this analysis is that the molecular interactions between the different polymer segments are well understood. In order to understand these interactions we have in an earlier work studied the temperature dependence of the phase equilibria in the system poly(ethylene glycol)/dextran/water.[5] In the present work we have continued from a preliminary report[6] and have studied how the cloud point temperature for an aqueous poly(ethylene glycol) (PEG) solution changes when small amounts of low molecular weight saccharides are added. Our studies show that the different saccharides show large differences in how they effect the PEG solutions. Further the cloud point changes and the phase diagram are well described with model calculations for the system poly(ethylene glycol)/glucose/water.

Materials and Methods

Poly(ethylene glycol) (trade name PEG20000) with a molecular weight of 17000–20000 and all the saccharides were obtained from Serva, Heidelberg, FRG. Before stock solutions were prepared, the polymer was completely dried over phosphorous pentoxide in a vaccum desiccator for at least one week. All samples were prepared with Millipore purified water.

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The phase transitions were detected visually in a closed glass tube and the temperature was controlled by immersion in an oil bath. The temperature was recorded with a copper constantan thermocouple placed in the sample. The phase transition temperature reported is the temperature when the last trace of cloudiness disappeared when lowering the temperature and entering a one-phase region from a two-phase region. The temperature was changed at an approximate rate of 2 °C/min and the recorded phase transition temperatures were reproducible to within less than 0.3 °C.

The phase diagram for glucose and PEG at 90 °C was determined in the following way. Suitable amounts of water and stock solutions were weighed into a test tube and mixed thoroughly. The sealed test tubes were equilibrated in a thermostated water bath and after mixing at the equilibration temperature the samples were kept in the water bath until the phases were totally separated.

Samples from the separated phases were taken for analysis of their polymer content. The glucose concentration was determined by optical rotation on a JASCO DIP 360 polarimeter and the total solute concentration, and thereby indirectly the poly(ethylene glycol) concentration, was determined by freeze-drying a weighed sample.

Results and Discussion

Like a number of nonionic surfactants and water-soluble polymers, poly(ethylene glycol)s (with a degree of polymerization exceeding about 50) have a lower critical solution temperature (LCST) in water.[7] The existence of a LCST means that a solution phase separates at temperatures over this point. The location of the LCST, often called the cloud point, is dependent on both the polymer concentration and the degree of polymerization. For poly(ethylene glycol) the cloud point is around 180 °C for the lower molecular weights, decreases with increasing molecular weight, and reaches a limiting lowest value of approximately 95 °C for molecular weights of 200 000 or more.[8]

In the literature there exist a lot of experimental studies of how the cloud points of both poly(ethylene glycol)s and other clouding nonionic polymers change when a third component is added to an aqueous solution.[9-11] In the case of poly(ethylene glycol) an overwhelming part of the studies deal with the effects when the third component added is an electrolyte.

In this study we have examined how the cloud point is changed by addition of small amounts of different uncharged saccharide molecules. The aim of the study has been to get a better understanding of the molecular interactions, which we stated in earlier work were responsible for the phase separations in both the binary poly(ethylene glycol)/water system and the ternary poly(ethylene glycol)/dextran/water system.[2,5,12]

In order to simplify the comparison between the different cloud point curves, all measurements are made on solutions that are prepared from stock solutions with a constant poly(ethylene glycol)/water w/w ratio of 0.100. To such a stock solution, which has a cloud point of 111.7 °C, different amounts of a third component is added and the cloud point is measured in the manner described above. When the change in cloud point from such an experiment is plotted against the weight/weight ratio of added third component and poly(ethylene glycol), the result is found to be linear in all our studied systems over a large range of concentration.

The most commonly used system for aqueous two-phase partitioning techniques in biochemistry is the poly(ethylene glycol)/dextran/water system, but other polysaccharides also show incompatibility with poly(ethylene glycol).[1,2,13] Since glucose is the monomer unit in dextran as well as in starch and cellulose, it seems natural to start with a rather extensive discussion of the poly(ethylene glycol)/glucose/water system.

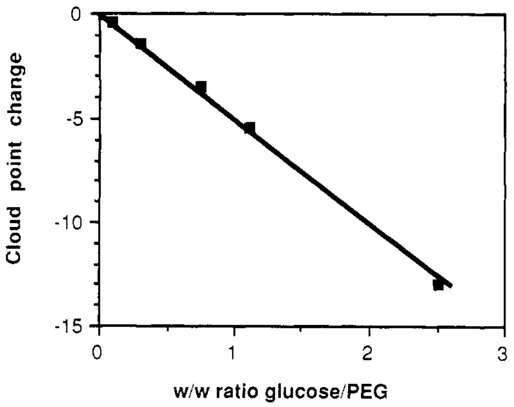

Figure 1. Cloud point as a function of the weight fraction glucose/PEG20000 for solutions with a constant weight ratio water/PEG20000 of 10.0.

Figure 1 shows how the cloud point of a 10.0 % w/w stock solution of poly(ethylene glycol) is changed on addition of glucose. As seen from the figure the depression of the cloud point is not that strong and to achieve a cloud point depression of 10 °C the glucose/poly(ethylene glycol) ratio has to be 2.1 (see Table III). Compared to the effects shown for addition of some electrolytes, this is a rather small effect.[14]

In ref 6 we gave an interpretation of cloud point depressions in poly(ethylene glycol) solutions when saccharides are added. In that work we suggested that the decrease was the result of the effective interaction between poly(ethylene glycol) and glucose being more repulsive than the interaction between glucose and water. The result of such a situation is that when the system separates into two phases, the glucose will predominately distribute to the polymer poor phase, thus lowering the chemical potential of the water and facilitating the phase separation so that it can take place at a lower temperature.

To test whether this picture was correct or not a phase diagram for the poly(ethylene glycol)/glucose/water system was determined at 90 °C. The phase diagram in Figure 2 and the composition of the separated phases in Table I show that the assumption of enrichment of the glucose in the polymer poor phase was correct.

The phase diagram is in principle similar to a typical polymer/polymer/solvent phase diagram. When compared to a poly(ethylene glycol))/dextran/water phase diagram, there are a few differences to be pointed out. First, there has to be a much higher solute concentration in the glucose case than in the dextran case before any phase separation takes place, and the temperature has also to be considerably higher. Second, even at high poly(ethylene glycol) content the amount of glucose in the poly(ethylene glycol) rich phase is not negligible, whereas dextran is almost exclusively found in the poly(ethylene glycol) poor phase. The explanation for both these effects is to be found in that the loss in entropy when a component is enriched in one phase is more pronounced in the case of free glucose molecules than in the case where the glucose molecules are monomers in a dextran polymer chain.

It is possible to analyze the effects of glucose additions within a model we have proposed for the temperature dependence of the phase equilibria in a poly(ethylene glycol)/dextran/water system. For a more detailed description of the model and model calculations see ref 5.

The model is based on statistical mechanical mean-field theory for the clouding phenomenon of poly(ethylene glycol) in water that one of us has developed from quantum

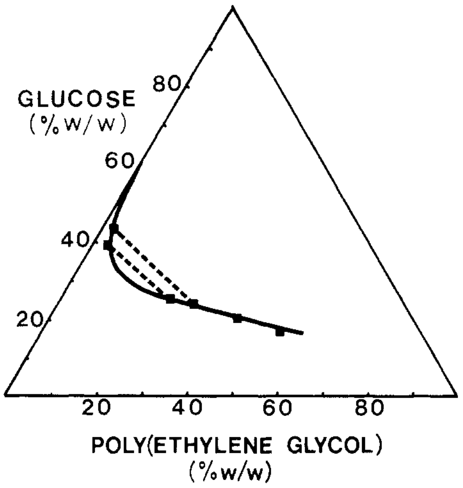

Figure 2. Experimental phase diagram for the system PEG20000/glucose/water at 90 °C. Experimental values from Table I.

Table I
Coexisting Phases in the Two-Phase Region at 90 °C[a]

bottom phase, % w/w		top phase, % w/w	
PEG20000	glucose	PEG20000	glucose
	53.0	52.2	16.4
	48.4	41.4	19.7
2.6	42.9	29.5	23.9
3.2	38.8	23.8	25.8

[a] Experimental error ±0.1% w/w.

chemical calculations of 1,2-dimethoxyethane.[12,15] In that description the emphasis is put on the conformational equilibria of the poly(ethylene glycol) chain. Since it is possible to have different dihedral angels around the C–C and the C–O bonds in the poly(ethylene glycol) chain, there are a vast number of different conformations with different dipole moment and different energy. At ordinary temperatures there is a low-energy polar conformation dominating and at higher temperatures there are several less polar conformations of higher energy populated. In the model an average is made over these less polar conformers.[12] As the temperature is increased the less polar conformations become more populated, making the polymer less hydrophilic and ultimately causing the phase separation.

A change in the local conformation will not only affect the interaction with water (w_{12}) but also the interaction with the other polymer's segments (w_{23}). Assuming a more repulsive interaction between the nonpolar conformers and dextran than between the polar ones and dextran implies an effective interaction parameter w_{23}(eff) that increases with temperature, that is when the proportion of less polar conformations is increased.

On the basis of these ideas we have combined the Flory–Huggins model, which is a mean-field model, of the two polymer system of ref 16 with the more detailed model of the poly(ethylene glycol)/water system of ref 12 to obtain the following expression[5] for the energy (or enthalpy when the system is assumed incompressible)

$$U = M_0\{w_{1L}P\phi_1\phi_2 + w_{1H}(1 - P)\phi_1\phi_2 + w_{13}\phi_1\phi_3 + w_{L3}P\phi_2\phi_3 + w_{H3}(1 - P)\phi_2\phi_3 + w_{LH}P(1 - P)\phi_2\phi_2 + w_{HH}(1 - P)(1 - P)\phi_2\phi_2/2\} \quad (1)$$

The corresponding expression for the entropy is

$$S/R = -M_0\{\phi_1 \ln(\phi_1) + (\phi_2/M_2)\ln(\phi_2) + (\phi_3/M_3) \times \ln(\phi_3) + \phi_2[P\ln(P) + (1 - P)\ln((1 - P)/F)]\} \quad (2)$$

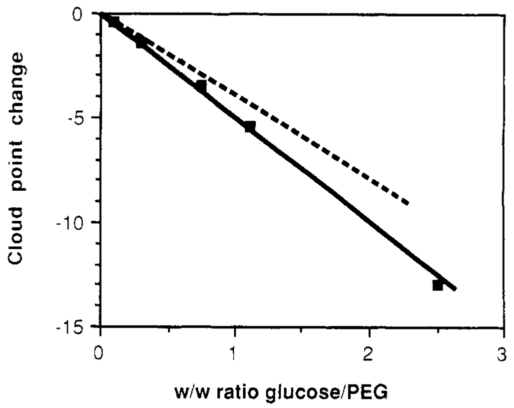

Figure 3. Comparison of the calculated and the experimental cloud point as a function of the weight fraction glucose/PEG20000 for solutions with a constant weight ratio water/PEG20000 of 10.0. The model system is defined in Table II.

The different components are 1, water; 2, PEG; and 3, dextran. Index L stands for the polar "low-temperature" form of PEG and H for the less polar "high-temperature" form of PEG. F, which is the statistical weight of the high-temperature form relative to that of the low-temperature form, has been given the value 8, and P is the fraction of the low-temperature form of PEG. M_0 is the total size of the lattice, and M_i and ϕ_i are the degree of polymerization and the volume fraction, respectively, for species i. The interaction energy parameter characterizing the interactions between a segment of type i and a segment of type j is denoted w_{ij} and is related to the ordinary χ parameter through

$$\chi_{ij} = w_{ij}/RT \quad (3)$$

Even though the model is developed for a polymer/polymer/solvent system it is possible to use it for calculations on the poly(ethylene glycol)/glucose/water system. The reason for this is that the glucose molecules are comparable with monomer units in a polymer chain at concentrations over C^*. That is, the glucose monomers can be treated as randomly distributed in space.

In figures 3 and 4 the results of the model calculations are shown together with the experimental results. It is worthwhile to mention something about the interaction energies used in the calculations (see Table II). In the previous work[5] we have used the same interaction energies that were used in the original work by Karlström for the binary system poly(ethylene glycol)/water. This is not possible to do in this case for the following reasons. A 10% w/w solution of Karlström's "model" poly(ethylene glycol) with a molecular weight of 20 000 has a cloud point of around 95 °C.[12] This is somewhat too low compared to the experimentally found cloud point of such a solution. The cloud point for the real poly(ethylene glycol) PEG20000 is found in our study to be 111.7 °C for a 10.0% w/w solution. This discrepancy did not matter in the previous studies since most of the calculations were performed at temperatures other than the cloud point temperature. In this case, however, we are interested in precisely that temperature and thus have to modify our interaction energies in some extent. The simplest way to get around this problem is to scale up all poly(ethylene glycol) interactions in a way that raises the cloud point to 111.7 °C. Thus all interaction parameters are multiplied by (111.7 + 273.15)/(95 + 273.15). The only effect such a procedure has on the binary phase diagram is that it is moved up in temperature. With these scaled interaction energies for the poly(ethylene glycol) interactions, it is

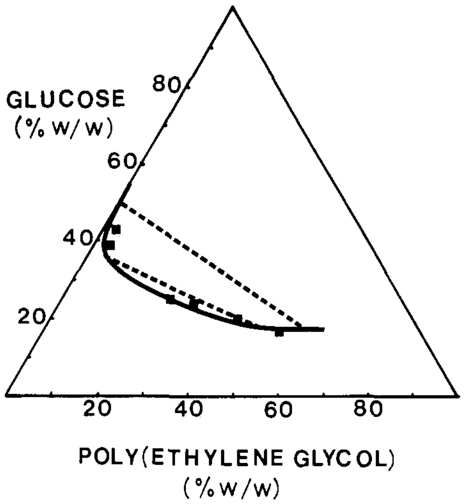

Figure 4. Calculated binodal curve (—) with examples of tielines (- - -) and experimental points (■) for the system PEG20000/glucose/water at 90 °C. The model system is defined in Table II.

Table II
Theoretical Model System

molecular weights	poly(ethylene glycol), 19800 g/mol
	glucose, 180 g/mol
interactions energies	w_{1L} = 683.8 J/mol
	w_{1H} = 11188 J/mol
	w_{13} = 5000 J/mol
	w_{L3} = 6320 J/mol
	w_{H3} = 18500 J/mol
	w_{LH} = 6670 J/mol
	w_{HH} = 10682 J/mol

possible to get a reasonable fit for both the phase diagram at 90 °C and the cloud point depression for the poly(ethylene glycol) solutions. The different glucose interaction energies are to some extent to be regarded as fitting parameters and should not be taken too literally.

However, the fit between experimental and calculated phase diagram in Figure 4 is not as good as the result in our previous works on poly(ethylene glycol)/dextran/water systems.[5] The explanation for this is, to some extent, to be found in the fact that we do not take into account the different sizes of a poly(ethylene glycol) monomer, a glucose molecule, and a water molecule. The effects of this approximation become more important for free glucose units than for glucose monomers linked together in a polymer chain.

When we then add a disaccharide instead of a monosaccharide the expected result is that the cloud point decrease will be more pronounced since the loss in entropy when the saccharide is enriched in one phase will be less per monomer unit in the disaccharide case than in the monosaccharide case. We have studied the effect on the cloud point upon addition of three different disaccharides. The studied disaccharides are maltose, cellobiose, and isomaltose. All three disaccharides consist of two glucose molecules linked together with different glycosidic bonds. The glycoside bonds are α-1,4, β-1,4, and α-1,6 for maltose, cellobiose, and isomaltose, respectively. The polymers corresponding to these dimers are starch, cellulose, and dextran.

In Figure 5 the cloud point depressions for additions of the three disaccharides to a 10.0 w/w poly(ethylene glycol) solution are shown. As seen from the figure the effects of maltose and cellobiose on the cloud point are identical (at least within experimental accuracy). Compared to the effects in Figure 1, the addition of maltose and cellobiose decreases the cloud point somewhat more than glucose dose. The difference is small but experimentally observable. In the case of addition of isomaltose the effect on the cloud point is much larger than the effect from addition of 1–4-linked disaccharides. Already in the dimers, the difference between dextran and starch in two-phase forming ability with poly(ethylene glycol) is established.

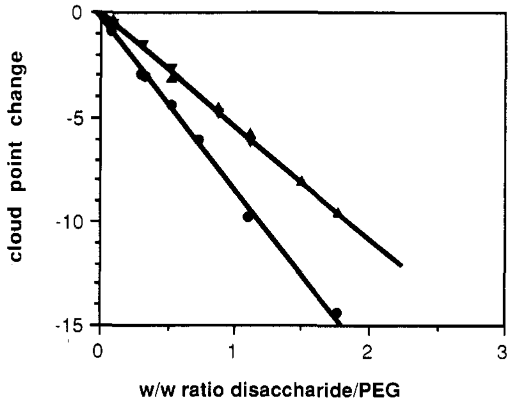

Figure 5. Cloud point as a function of the weight fraction glucose dimer/PEG20000 for solutions with a constant weight ratio water/PEG20000 of 10.0. The dimers are maltose (▲), cellobiose (▼), and isomaltose (●).

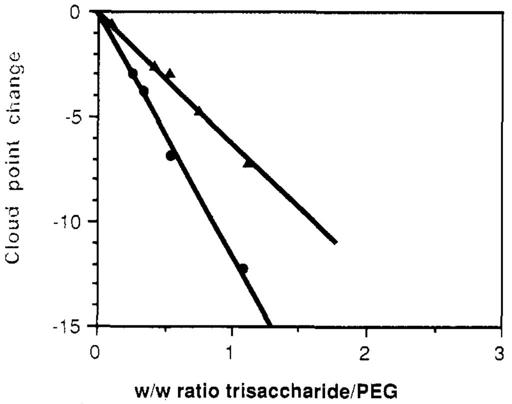

Figure 6. Cloud point as a function of the weight fraction glucose trimer/PEG20000 for solutions with a constant weight ratio water/PEG20000 of 10.0. The trimers are maltotriose (▲) and isomaltotriose (●).

Even if the cloud point depression in a poly(ethylene glycol) solution was found to be larger for the case of added maltose and cellobiose than in the case of glucose, the difference was very small. The effect was much more clearly seen when the added disaccharide was isomaltose. The trend that a 1–6-linked saccharide is much more effective as a cloud point depressor than a 1–4-linked saccharide is even more pronounced for trisaccharides. In Figure 6 the result of adding the two trisaccharides maltotriose and isomaltotriose to a 10.0% w/w poly(ethylene glycol) solution is shown. As can be seen both from the Figures and Table III the difference between the addition of the trimer and the dimer of one type, to the poly(ethylene glycol) solution, is larger than the difference between addition of the same dimer and the monomer glucose. Also for the trisaccharides the 1–4-linked maltotriose does not decrease the cloud point nearly as much as the 1–6-linked isomaltotriose. The differences between the 1–6-linked saccharides and the 1–4-linked saccharides are so great that addition of isomaltose lowers the cloud point more than an equal amount of the trisaccharide maltotriose does.

Why is a 1–6-linked linear saccharide much more effective in depressing the cloud point in a poly(ethylene

Table III
Amount of Saccharide Needed for a Cloud Point Decrease of 10 °C When Added to a 10.0% w/w Poly(ethylene glycol) Stock Solution[a]

saccharide	saccharide/PEG, w/w
glucose	2.1
cellobiose	1.9
maltose	1.9
isomaltose	1.2
maltotriose	1.6
isomaltotriose	0.86

[a] The amount of saccharide is given as the weight/weight ratio saccharide/poly(ethylene glycol) in the final solution.

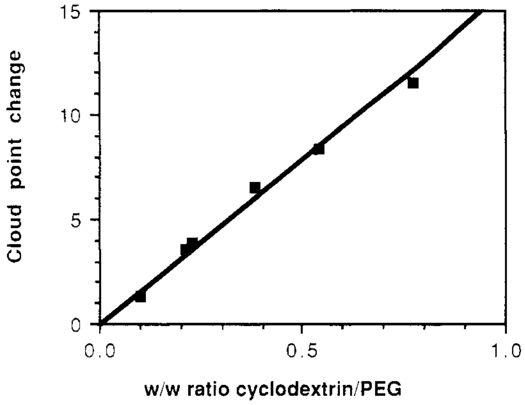

Figure 7. Cloud point as a function of the weight fraction β-cyclodextrin/PEG20000 for solutions with a constant weight ratio water/PEG20000 of 10.0.

glycol)/water system than a 1–4-linked linear saccharide with the same number of glucose units in it? The answer to this question consists of many parts but to some extent a tentative explanation can be given with arguments from conformational energy calculations of disaccharides. For maltose and cellobiose, where the two glucose rings are connected via an oxygen atom and two ring carbons, such a calculation shows the possibility of forming an intramolecular hydrogen bond.[17] One has to keep in mind that these low-energy conformations with intramolecular hydrogen bonds calculated for crystal conformations are not to be regarded as locked conformations in solution. However, there is experimental support that the intramolecular hydrogen-bonded conformations, on average, are populated to a higher extent than other conformations in solution.[17]

The result of intramolecular hydrogen bonding will be that the maltose and cellobiose cannot develop, on average, as many hydrogen bonds with water as the isomaltose can. This means that the 1–4-linked disaccharides, being less able to interact with the surrounding solvent, will be less hydrophilic than isomaltose. That is, cellobiose and maltose will effectively interact more favorably with the nonpolar poly(ethylene glycol) and thus also decrease the cloud point less than the isomaltose does. This effect will also be increased by the fact that the 1–6 glycosidic link consists of both an oxygen and a carbon between the glucose rings, making isomaltose better suited to interact with the surrounding solvent than the more dense 1–4-linked structures can.

To test these tentative explanations, we have also studied how the cloud point in poly(ethylene glycol) solutions is changed when β-cyclodextrin is added. β-Cyclodextrin is a nonreducing cyclic oligosaccharide with seven α(1–4)-linked glucose molecules. The β-cyclodextrin molecule has a number of intramolecular hydrogen bonds and has a surface and cavity that are slightly nonpolar.[18,19] The suspected result of adding such a molecule to a poly(ethylene glycol) solution is that there should be a lesser decrease in the cloud point. This result is confirmed in Figure 7 and, as seen, the cloud point even increases with the addition of β-cyclodextrin. The effects of the intramolecular interactions are here so pronounced and the β-cyclodextrin is so hydrophobic in nature that it is probably enriched in the top phase and thus increases the cloud point temperature.

Acknowledgment. This work was supported by grants from the National Swedish Board for Technical Development (STU).

Registry No. PEG, 25322-68-3; glucose, 50-99-7; maltose, 69-79-4; cellobiose, 528-50-7; isomaltose, 499-40-1; maltotriose, 1109-28-0; isomaltotriose, 3371-50-4; β-cyclodextrin, 7585-39-9.

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