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Impact of Water on the Miscibility of DAB-dendr-$(NH_2)_{64}$ and Benzene

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ABSTRACT: The miscibility of the fifth-generation 1,4-diaminobutane poly(propyleneimine) (DAB-PPI) dendrimer and benzene was investigated. The liquid−liquid phase transitions in mixtures containing from 0.02 to 0.26 weight fraction of the dendrimer were determined. It was shown that the miscibility of the dendrimer and benzene depend to a great extent on the water content in the system. The binary system dry dendrimer + dry benzene does not show a liquid−liquid phase split. The addition of small amounts of water (as small as 0.06 wt %) to the system induces complex phase behavior. The liquid−liquid phase transitions in the ternary systems DAB-PPI dendrimer + benzene + water (0.06 wt %) are accompanied by unusual changes in the properties of the coexisting phases. The influence of water on the ^{1}H NMR spectra of the fifth-generation DAB-PPI dendrimer dissolved in benzene was also investigated.

Introduction

Highly branched dendritic macromolecules with a complex architecture and a large number of surface functional groups are a relatively new class of polymer. The two main classes of dendritic macromolecules are random hyperbranched polymers and dendrimers. A variety of possible applications make the phase behavior of their solutions an important issue. Random hyperbranched polymers are in fact mixtures of species differing in molecular weight and the degree of branching. Mixtures of hyperbranched polymers and small-molecule solvents show liquid−liquid phase behavior similar to the phase behavior of linear polymer solutions.[1]

In contrast to hyperbranched polymers, dendrimers are uniform, well-defined, highly symmetric branched macromolecules. Thus, in principle a mixture composed of the dendrimer and solvent is strictly a binary system, in which the liquid−liquid phase behavior should resemble that observed in small-molecule systems. This supposition cannot be verified since, according to our knowledge to date, there have been no experimental liquid−liquid phase equilibria in binary dendrimer solutions reported in the literature. Theoretical considerations on phase behavior in dendrimer solutions suggest that depending on the interaction energies between solvent molecules and the dendrimer both an upper and a lower miscibility gap are encountered.[2,3] The scheme of phase behavior in dendrimer solutions emerging from molecular simulations is similar to that observed in other asymmetric systems.[4] Modeling of phase equilibria in these systems is focused on the influence of branching and the nature of the surface end groups on the position and the extent of the miscibility gap. The unimolecular micelle-like behavior of dendrimer molecules is a manifestation of unusual properties which are not encountered in linear polymers. The dendrimers' capability of molecular inclusion may influence the liquid−liquid phase behavior of their solutions. This may be particularly prominent when trace components present in the system modify the structure of the dendrimer molecules. This type of effect is not possible for linear macromolecules.

This study reports experiments showing the unusual influence of traces of water on liquid−liquid phase behavior in the fifth-generation DAB-PPI dendrimer + benzene system. The DAB-PPI dendrimer molecule consists of a core 1,4-diaminobutane molecule and covalently bound dendrons built with propyleneimine units. The nitrogen atoms in the core are separated by four CH_2 groups, while three CH_2 groups separate all other nitrogen atoms from each other. The outermost shell of the fifth-generation DAB-PPI dendrimer ends with 64 primary amine functional groups in the periphery. The DAB-PPI dendrimer molecule is hydrophilic.

Experimental Section

Liquid−Liquid Equilibria. To ensure a water- and oxygen-free environment in the phase equilibrium experiments, the synthetic ampule method was used. This method makes direct observations of the phase transitions possible and at the same time provides an easy and reliable way of controlling the amount of water in the system. The phase equilibrium experiments were performed for the water-free binary system DAB-PPI + benzene, the system dry dendrimer + benzene saturated with water at 299 K, and the ternary system DAB-PPI + benzene + 1 μL of water.

The procedure for preparation of water-free ampules was as follows: First, the empty ampule was connected to a high-vacuum line, heated, and evacuated for about 24 h to remove water adsorbed at the glass surface. Then a sample of DAB-PPI was placed in the ampule. Loading of the dendrimer was performed in an atmosphere of dry nitrogen. The ampule was again connected to the high-vacuum line and evacuated. It was kept at 343 K under vacuum for about 24 h to remove water from the dendrimer. The evaporation of water is an important step since commercially available DAB-PPI dendrimer contains from 0.1 to 4 wt % of water.[5] Benzene was dried with metallic sodium and degassed by repeated crystallization and evacuation. Then it was transported under vacuum directly to the ampule. Next the ampule was sealed. The amounts of the dendrimer and benzene were established by weight.

In the case of benzene saturated with water as the solvent the procedure of filling the ampules with the dendrimer was the same as before. The solvent was added to the ampule with a syringe in a nitrogen atmosphere, and the ampule was sealed.

The ampoules with 1 μL of water were prepared according to the following procedure: First the ampule was filled with dry dendrimer and dry benzene according to the procedure described

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above. Next, 1 μL of water was added to the ampule with the syringe, and the ampule was sealed.

For the measurement of phase transition the ampule was placed in a flow-through glass jacket. A small glass spin bar inside and variable position of the ampule in the jacket ensured very efficient stirring. The jacket was attached to an external fluid thermostat with temperature control within 0.1 K. The temperature of the fluid in the jacket was measured with the RTD probe (SYSTEMTEKNIK, model S 1220). The experiments were carried out in a quasi-isothermal regime, and the phase transitions were detected visually through the optical system.

NMR. All NMR samples were prepared according to the same procedure as was used for the preparation of the ampoules in the phase equilibria experiments. Benzene-d_6 used in NMR experiments as the solvent was dried with sodium and was transported under vacuum to the NMR ampules. NMR spectra were obtained on a Bruker DRX Avance 500 MHz.

Materials. The fifth-generation DAB-PPI dendrimer was purchased from Aldrich Chemical Co. The vials containing dendrimer were placed in a desiccator and kept in a nitrogen atmosphere during the whole period of the study. Mass spectra of fresh dendrimer and dendrimer used in phase equilibrium experiment were determined. No differences in the appearance of these spectra were observed, which is an indication of the stability of the DAB-PPI molecules in our experiments. The dendritic purity estimated from these spectra was about 25%. The structure of the spectrum was very similar to that presented by Hummelen et al.[6] The spectrum and its description can be found in the Supporting Information.

Benzene (C_6H_6) (>99.8 wt %) and deuterated benzene (C_6D_6) (>99.8 wt %) were purchased from CHEMIPAN, Poland.

Results

Liquid−Liquid Equilibria in DAB-dendr-$(NH_2)_{64}$ + Benzene + Water System. The first experiments concern the miscibility of dry DAB-PPI dendrimer and dry benzene. Six binary systems of dry DAB-PPI + dry benzene were investigated. The mixtures contained 0.0792, 0.0865, 0.0909, 0.0996, 0.1201, and 0.1760 weight fractions of the dendrimer. In all samples that did not contain water the solute dissolved very easily in benzene without excessive heating, and the solution was clear without fuzzy particles. No liquid−liquid phase split was observed from the crystallization temperature of benzene (278.65 K) up to 370 K (upper temperature boundary of our equipment). The crystallization temperatures obtained by cooling binary mixtures are always lower than the melting temperature of benzene due to the supercooling effect. No systematic shift in the crystallization temperatures of the mixtures with the concentration of the dendrimer was observed.

In the next stage, mixtures of dry dendrimer and benzene saturated with water at 299 K were investigated. According to Sørensen and Arlt[7] at this temperature benzene contains about 0.07 wt % water. The amount of water in benzene was confirmed by the Fisher method. Ten mixtures containing benzene saturated with water and dry dendrimer were prepared.

Surprisingly, the phase behavior in the system DAB-PPI dendrimer + benzene after addition of a trace of water changed dramatically. A very unusual picture of phase behavior in this, in fact, ternary system emerged. The results of the measurements are presented in Table 1 and are shown in Figure 1. The concentration of water in the samples changed depending on the amount of benzene added. Nevertheless, the differences in water concentration between samples were small. The average concentration of water in this system was 0.06 wt %.

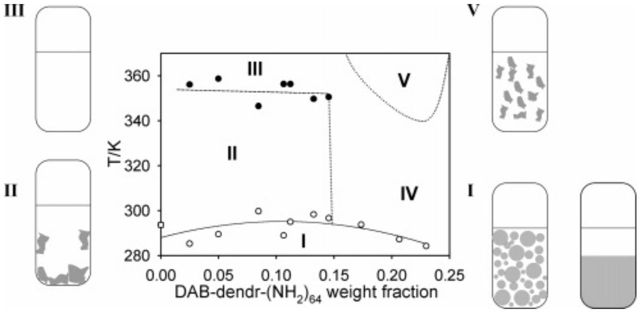

Figure 1. Phase behavior for the system dry DAB-PPI + benzene saturated with water at 299 K.

Table 1. Phase Equilibria for the System DAB-PPI + Benzene Saturated with Water at 299 K (w = Weight Fraction of the Dendrimer)

w	T/K	w	T/K
	Cloud Point		
0.0249	356.2	0.1324	349.8
0.0499	358.7	0.1454	350.6
0.0846	346.6	0.1735	293.8
0.1064	356.4	0.2065	287.3
0.1122	356.4	0.2298	284.3
	"Ball" Phase		
0.0000	293.8	0.1324	298.4
0.0249	285.4	0.1454	296.8
0.0499	289.6	0.1735	293.9
0.0846	299.9	0.2065	287.3
0.1064	289.0	0.2298	284.3
0.1122	295.1		

For mixtures with a dendrimer concentration of up to about 0.15 weight fraction the following phase transitions were observed. At temperatures higher than 360 K the system was homogeneous (area III in Figure 1). In the figure the appearance of the content of the ampule in all areas of the phase diagram is shown schematically. In all cases the upper, the gas phase consisted mainly of benzene and was always present in the system. On temperature decrease, at about 360 K, a cloud point was observed. The behavior of the system at the onset of phase separation was very similar to that observed in other polymer solutions. However, immediately after this initial moment a significant difference was observed. The new phase was very viscous, and it formed large clusters sticking to the walls of the ampule and to the stirring bar. The area of the "sticky phase" in the phase diagram is marked as II. It seems that the phase appearing at 360 K was mainly composed of the dendrimer. On further temperature lowering at about 300 K the sticky phase liquefied. In this region, a second liquid phase formed on mixing regular balls. The area of the "ball" phase in the phase diagram is marked as I.

In Figure 2 two photos of the "ball" phase just after mixing (a) and after full separation of the phases (b) are presented. It seems that the surface tension of the lower, dendrimer-rich phase is high, and the spherical pieces of this phase once formed are not eager to join. When the two phases are separated, it is difficult to see the phase boundary since the refractive indices of the coexisting liquid phases have similar values.

The temperature of liquefaction of the sticky phase is well-defined. However, by no means should the transformation of the sticky phase into the "ball" phase be treated as the phase transition. It seems that at 360 K the phase rich in the dendrimer precipitates while most of the water remains in the benzene. At about 300 K water separates from the benzene and dissolves the dendrimer sticky phase. The open square in Figure 1 indicates the phase separation in the binary system benzene saturated with water at 299 K measured with our ampule method. The transition temperature agrees reasonably well with the temperature of the appearance of the "ball" phase in the ternary system.

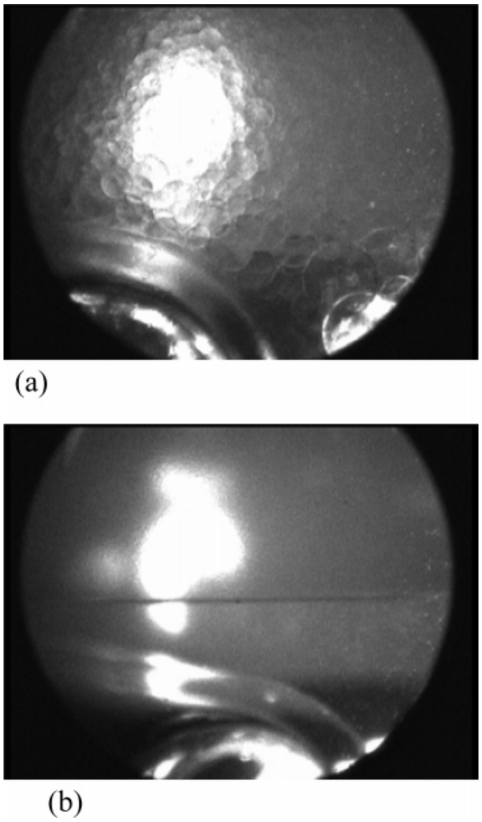

Figure 2. Separation into two phases of the system DAB-dendr-$(NH_2)_{64}$ + benzene + 1 μL H_2O at 301.95 K.

As has already been mentioned, experiments for the system dendrimer + benzene saturated with water described up to now were performed on a temperature decrease. The detection of the observed transitions on temperature increase was practically not possible. In the case of the I → II transition the "ball" phase on heating did not disappear but changed its properties slowly. As the temperature was increased, droplets of the dendrimer rich phase lost their regular ball shape and at the same time their viscosity increased. Finally, this phase became so viscous that it started to stick to the walls of the ampule and to the stirring bar. The temperature interval for this process to occur was quite wide, and it was difficult to assess the temperature of this transformation. This process was a result of the transfer of water from the dendrimer-rich phase to the benzene-rich phase. It seems that removal of water is controlled by diffusion, and that is why the process is extended in time and temperature. In the opposite direction (II → I) the phase rich in water separated from benzene, and this process was very fast. The mixing of the sticky phase and water takes more time, and this was possibly the reason for the scatter of the data.

The temperature of the dissolution of the sticky phase (II → III transition) was also difficult to determine. Vigorous stirring of the contents of the ampule was needed to dissolve the dendrimer phase stuck to the glass. Close to the dissolution temperature a small amount of the dendrimer phase lying on the ampule's walls was difficult to observe. Thus, in fact to be sure that all the dendrimer phase had dissolved, overheating of the system was necessary.

For mixtures with a dendrimer concentration above 0.15 weight fraction different phase behavior was observed. This boundary in the phase diagram is not sharp. The transition from area II to area IV occurred gradually, and the vertical dashed line in Figure 1 indicates only an approximate position of the boundary. As is seen in Figure 1, the area I ("ball" phase") is common for both regions of the phase diagram. However, the two-phase system with overall dendrimer concentration greater that 0.15 weight fraction homogenized on heating. At temperatures just above the line drawn through the open circles the dendrimer seems to be completely dissolved. If there were any clusters of the dendrimer phase in the ampule, it was not possible to see them with the magnification of our optical system. However, on further temperature increase small pieces of the dendrimer phase appeared (area IV in Figure 1). In area IV as the temperature was increased the amount of the dendrimer phase increased. Thus, as before, the dendrimer-rich phase appeared, but this time it did not stick to the glass. The clusters of this phase flowed freely in the benzene-rich phase. Apparently the dendrimer-rich phase changed its adhesive properties toward glass. At higher temperatures the amount of this phase stabilized (area V in Figure 1). There was no real transition between areas IV and V; the difference was only quantitative. Lowering the temperature of the systems from about 360 K resulted in a slow disappearance of the dendrimer-rich phase, and no cloud point was observed. Just before the appearance of the "ball" phase the solution seemed to be homogeneous.

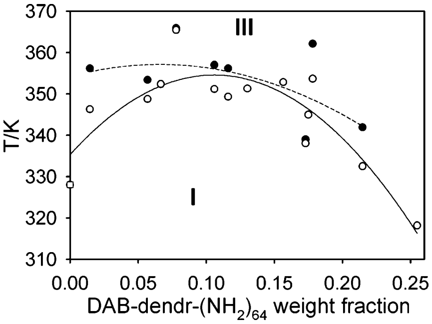

Figure 3. Phase behavior of the system DAB-PPI + benzene + 1 μL of water.

Table 2. Phase Equilibria for the System DAB-PPI + Benzene + 1 μL of Water (w = Weight Fraction of the Dendrimer)

w	T/K	w	T/K
	Cloud Point		
0.0146	356.2	0.1302	351.3
0.0569	353.4	0.1565	352.8
0.0667	352.4	0.1729	339.0
0.0779	365.9	0.1749	345.0
0.1059	357.0	0.1782	362.1
0.1161	356.2	0.2147	341.9
	"Ball" Phase		
0.0000	328.1	0.1302	351.3
0.0146	346.3	0.1565	352.8
0.0569	348.8	0.1729	338.1
0.0667	352.4	0.1749	345.0
0.0779	365.5	0.1782	353.7
0.1059	351.2	0.2147	332.5
0.1161	349.3	0.2548	318.2

To investigate how the amount of water influences the phase behavior in the ternary system, the phase transitions in the system DAB-PPI dendrimer + benzene + 1 μL of water were measured. The concentration of water in the samples changed depending on the amounts of the dendrimer and benzene added. The differences in water concentration between samples were small. The average concentration was about 0.6 wt %, 10 times greater than in the mixtures containing benzene saturated with water. Fourteen samples containing 1 μL of water with different dendrimer content were prepared. The results of the measurements are presented in Table 2 and are shown in Figure 3. The increase of the amount of water significantly simplified the phase behavior in the DAB-PPI dendrimer + benzene + water system.

Table 3. Compositions of the Samples Used in NMR Experiments

no. of the sample	composition of the sample		concentration of dendrimer (wt %)	concentration of water (wt %)
	dendrimer	benzene		
1		dry	0	0
2		saturated with water at 299 K	0	0.07
3	dry	dry	12.52 (0.02 M)	0
4	dry	saturated with water at 299 K	14.57 (0.02 M)	0.06
5	raw (0.1−4 wt % water content)	dry	14.24 (0.02 M)	0.06−0.6

It seems that the cloud point transition was observed at the same temperature level, while the temperature at which the "ball" phase appeared was much higher than for systems with a lower concentration of water. In this case a direct transition from the homogeneous solution to the two-phase system ("ball" phase + benzene-rich phase) was observed. The dendrimer phase (II or V in Figure 3) was not detected in this case. Even if the cloud point was observed, the precipitation of water from benzene and the formation of the "ball" phase followed, so that the dendrimer-rich phase did not have the opportunity to form. The scatter of the data is a consequence of the kinetics of the phase separation. It should be kept in mind that in the narrow temperature range three processes are being dealt with: precipitation of the dendrimer phase, precipitation of water, and mixing of both phases.

NMR Spectra of DAB-dendr-$(NH_2)_{64}$ in Benzene. The NMR spectra of the fifth-generation DAB-PPI dendrimer were studied in deuterated benzene (C_6D_6) as the solvent. Five samples were prepared. Table 3 presents the samples compositions.

The ^1H NMR spectra of dry and saturated with water C_6D_6 (samples 1 and 2) were measured. Comparison of these spectra shows that signals from water occur at about 0.44 ppm. The spectra and their description can be found in the Supporting Information.

In Figure 4 the ^1H NMR spectrum of 0.02 M solution of dry DAB-PPI dendrimer in dry C_6D_6 is presented. There are 17 unique methylene groups in DAB-64, which are numbered from the core to the exterior of the dendrimer molecule (Figure 4a). For obvious reasons the spectrum for the generation 5 dendrimer is very similar to those for generation 3. The 750 MHz ^1H NMR spectrum of DAB-16 has been presented by Chai et al.[8] This spectrum has well resolved peaks from all methylene protons. In the spectrum of DAB-64 presented here the signals from metylene protons were not well resolved, possibly because of a lower frequency of the NMR apparatus (500 MHz) used and a difference in the size of the investigated macromolecules. Nevertheless, comparison of the NMR spectra of both generations makes it possible to assign group of signals in the spectrum of the DAB-64 dendrimer to the methylene groups. The assignment is shown in Figure 4b. The signal near 1.34 ppm is from volatile hydrocarbon impurities present in C_6D_6.

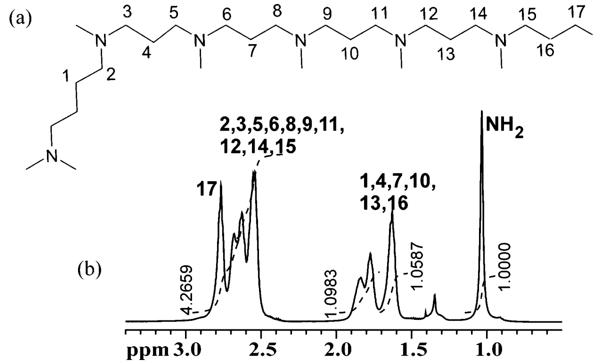

Figure 4. ^1H NMR spectrum of the system DAB-dendr-$(NH_2)_{64}$ + C_6D_6 (sample 3) at 303.15 K.

The aim of this study was to gain an understanding of the influence of water on the structure of the dendrimer molecules which could explain the unusual difference in the phase behavior of the DAB-dendr-$(NH_2)_{64}$ + benzene system and the same system after addition of small amounts of water. Water can influence the structure of the DAB-PPI molecule through an interaction with nitrogen atoms in primary and tertiary amine groups. Thus, well-resolved signals of the methylene groups and their precise assignment are not relevant for further discussion. However, assignment of a signal to the protons of the NH_2 group is of crucial importance. Chai et al.[8] showed that the NH_2 protons in the ^1H 1D-NMR spectrum of the DAB-16 in benzene have a chemical shift of 1.52 ppm and are buried under the resonance of the methylene protons of carbon 10 (the number of the carbon is from Chai et al.[8]). The exchangeable protons of the NH_2 group form one broad signal. Fortunately, in the spectrum of DAB-64 in benzene-d_6 obtained here the peak from the NH_2 protons was shifted upfield and was not broadened. The position of this peak near 1.03 ppm for the dry dendrimer + dry C_6D_6 system at 303.15 K (Figure 4b) placed it far from the methylene signals. The reason for the difference in appearance of the signals of the NH_2 protons of the DAB-PPI generation 5 presented here and the work of Chai et al.[8] comes mainly from the difference in dendrimer concentration. In this study spectra were obtained for solutions with a DAB-64 concentration 16 times lower than spectra investigated by Chai et al. Hydrogen bonds between terminal amine groups are responsible for the shift to a lower field and a broadening of the signal. Dilution of the system with an inert solvent results in breaking the intermolecular H-bonds and an increase in the shielding of the amine protons.

An unambiguous NMR resonance assignment to the protons of the NH_2 group can be established on the basis of the following facts. First of all, it is clear from the spectrum shown in Figure 4b that the integration ratio of methylene groups to amine groups (6.3:1) does not depend on temperature and is close to the value obtained from the structure of the dendrimer molecule (6.0:1). In Figure 5 the influence of temperature on the ^1H NMR spectrum of the dry DAB-64 dissolved in dry C_6D_6 is presented. It is evident that on temperature increase the NH_2 signal is shifted upfield by about 0.08 ppm and that its half-width diminishes. Both effects are characteristic for associating solutes. The positions of all other signals remain unchanged on temperature increase.

Figure 6 shows the 2D C,H-HSC NMR spectrum of DAB-64 in benzene at 303.15 K. This spectrum provides information on the direct bonding of carbon and hydrogen atoms. As can be seen, there is no direct coupling between the proton signal at 1.03 ppm and carbon signals. All these facts suggest that a peak at 1.03 ppm can be unambiguously assigned to the protons of the amine terminal groups.

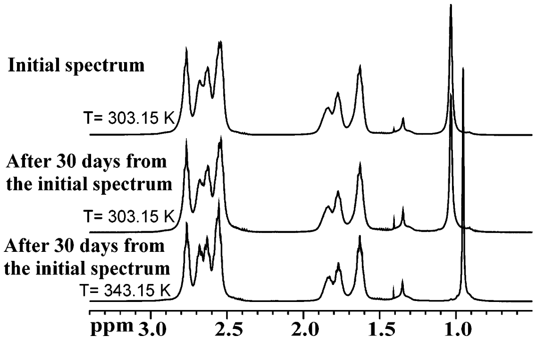

Figure 5. ^1H NMR spectra of the dry DAB-dendr-$(NH_2)_{64}$ dissolved in dry C_6D_6 (sample 3).

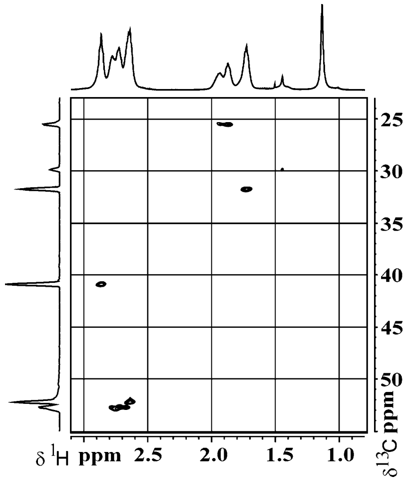

Figure 6. 2D C,H-HSC NMR spectrum of the system dry DAB-dendr-$(NH_2)_{64}$ + dry C_6D_6 (sample 3) at 303 K. 1D ^1H and ^{13}C NMR spectra are plotted on the top and the side, respectively.

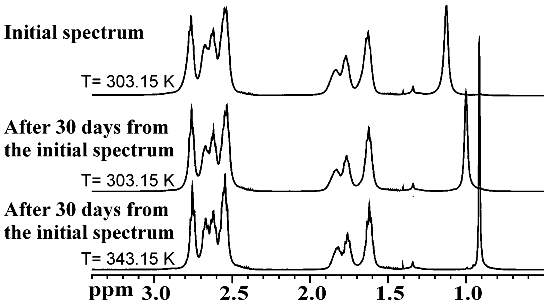

Figure 7. ^1H NMR spectra of the system dry DAB-dendr-$(NH_2)_{64}$ + C_6D_6 saturated with water at 299 K (sample 4).

Comparison of the ^{13}C NMR spectrum of DAB-64 with that for DAB-16[9] confirms that the proton signal near 1.34 ppm does not come from the dendrimer molecule.

In Figure 7 three spectra for the dry DAB-PPI dendrimer (generation 5) + C_6D_6 system saturated with water at 299 K are presented (sample 4 in Table 3). In the initial spectrum, obtained at 303.15 K the day after the sample preparation, the signal of the NH_2 protons lay at 1.12 ppm. The chemical shift of these protons was much higher than in sample 3 containing dry dendrimer and dry benzene. Apparently, the introduction of water to the system significantly decreased shielding of the terminal amine protons.

NMR spectra of sample 4 were acquired at two temperatures 30 days after the initial spectrum. Surprisingly at a temperature of 303.15 K, the peak from the NH_2 protons was near 1.00 ppm. It shifted upfield by about 0.13 ppm in comparison to the initial spectrum, and at the same time its half-width diminished. On temperature increase (to 343.15 K), the signal from the NH_2 protons shifted upfield by about 0.1 ppm and its half-width diminished. The positions of all other signals remained unchanged after 30 days. The ^1H NMR spectrum was also collected 60 days after the initial experiment. No further change of the chemical shift of the NH_2 protons in comparison to the measurements performed after 30 days was observed. The spectrum for the mixture without water (sample 3) collected 30 days after the initial measurements did not show any changes in the position of the amine protons (see Figure 5). Apparently the presence of water in the system made the difference.

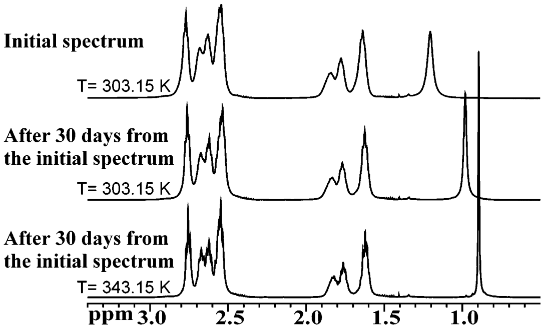

Figure 8. ^1H NMR spectra of the system raw DAB-dendr-$(NH_2)_{64}$ + dry C_6D_6 (sample 5).

In Figure 8 three spectra for the raw DAB-PPI dendrimer + dry C_6D_6 system (sample 5 in Table 3) are presented. The actual concentration of water in sample 5 was not known, since the exact concentration of water in the dendrimer obtained from Aldich was unknown. The only available information is that the dendrimer contained from 0.1 to 4 wt % water. Sample 5 homogenized on heating at about 308 K. On cooling the sample the onset of the phase transition was observed at about 302 K. Thus, it remained homogeneous at 303 K making the collection of the NMR spectra at this temperature possible. The temperature of phase transition indicated that the amount of water in sample 5 was slightly greater than 0.06 wt % and much lower than 0.6 wt % (see Figure 4).

In the initial spectrum of sample 5, obtained at 303.15 K the day after the sample preparation, the signal of the NH_2 protons lay at 1.20 ppm. The chemical shift of these protons was even higher than in sample 4 containing dry dendrimer and benzene saturated with water. The NMR spectra of sample 5 were also acquired at two temperatures 30 days from the initial spectrum. At a temperature of 303.15 K, the peak from the NH_2 protons shifted upfield to 0.98 ppm and its half-width diminished. The positions of all other signals remained unchanged after 30 days. The ^1H NMR spectrum was collected also 60 days from the initial experiment. No further change of the chemical shift of the NH_2 protons in comparison to the measurements performed after 30 days was observed. On temperature increase (to 343.15 K), the signal from the NH_2 protons shifted upfield by about 0.1 ppm and its half-width diminished.

Discussion

Liquid−Liquid Equilibria in DAB-dendr-$(NH_2)_{64}$ + Benzene + Water System. The results obtained seem to support the conjecture that in the temperature range investigated benzene is a good solvent for the DAB-dendr-$(NH_2)_{64}$ provided that there is no water in the system. This is in accordance with the fact that benzene is miscible with amines and as it is widely believed the character of the end groups dominates the physical properties of dendrimers with respect to solubility. The results presented clearly show that on addition of small amounts of water to a

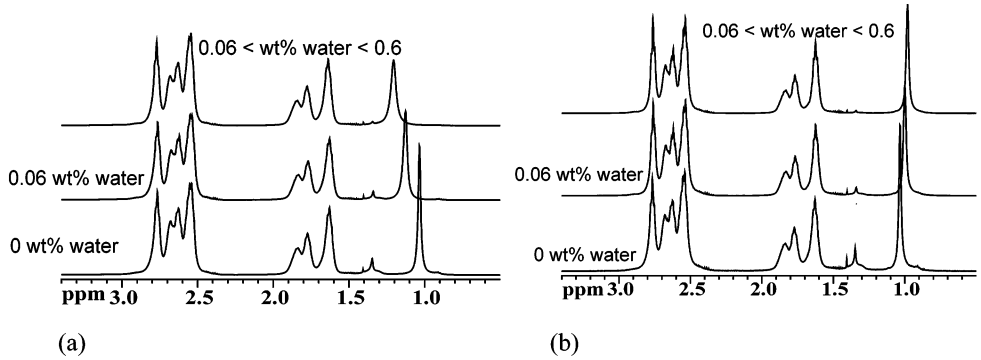

Figure 9. Influence of water on the ^{1}H NMR spectra for the system DAB-dendr-$(NH_2)_{64}$ + C_6D_6: (a) initial spectrum at 303 K; (b) spectra acquired at 303 K after 30 days from the initial spectra.

mixture of fifth-generation DAB-PPI dendrimer and benzene unusual phase behavior is observed. It seems that the observed phenomena are not a result of the transition from the binary to the ternary system. An excellent example of the influence of traces of water on the liquid−liquid equilibria of a binary small-molecule system was presented by Tveekrem and Jacobs.[9] They showed that an increase of water content in the methanol + cyclohexane system from 0.05 to 0.85 vol % shifted the two-phase region of about 30 K to higher temperatures. Although the effect was quite large, the nature of the phase behavior did not change. In this system the shift of the critical temperature was accompanied by changes in the critical composition and the critical exponent. The amount of water added to the binary DAB-PPI dendrimer + benzene system in our study was similar to Tveekrem and Jacobs' experiments. However, in our case a qualitative change in phase behavior was observed. After the addition of water the completely miscible system showed very complex phase behavior. In our opinion, the complexity of the phase behavior was a result of the nature of the solute molecules. The fifth-generation DAB-PPI dendrimer is capable of the inclusion of large guest molecules.[10,11] Small water molecules can enter the interior of the dendrimer even more so. They form hydrogen bonds with the surface primary amine groups. In this way the structure of the dendrimer molecules is modified. The interaction of the dendrimer−water clusters with benzene is different than the interaction of plain dendrimer molecules with the same solvent. This is indirectly visualized by the complete miscibility of the DAB-PPI dendrimer and dry benzene in contrast to the limited miscibility of the dendrimer and benzene saturated with water. From our experiments, it is evident that on temperature increase water is transferred from the dendrimer-rich phase to the benzene-rich phase. As a result, the amount of water molecules attached to the dendrimer molecules changes with temperature. A direct conclusion is that the topological conformation of the dendrimer−water complexes evaluates with temperature. This is a possible explanation for the complex phase behavior observed in the DAB−PPI dendrimer + benzene + water system.

NMR Spectra of DAB-dendr-$(NH_2)_{64}$ in Benzene. All spectra measured at 303 K for the three dendrimer solutions (samples 3−5) are presented together in Figure 9. The arrangement of the spectra emphasizes the influence of water. It should be stressed that the treatment of the mixtures enclosed in the NMR tubes was identical in all measurement runs. They were heated to 343 K and homogenized. Then the temperature was lowered to 303 K, and the samples were kept at this temperature until the NMR spectrum was measured (i.e., about 12 h).

Figure 9a show the spectra collected about 24 h after preparation of the samples. It is clear that the position of the NH_2 signal depends on the amount of water in the system. It seems that the effect is not a direct consequence of the interaction of water molecules with the amine terminal groups. The position of the averaged signal of the protons from NH_2 and H_2O would be proportional to the amount of water and would be shifted upfield toward the signal of water, i.e., 0.44 ppm. In Figure 9a the peak of the NH_2 protons is not only shifted to the opposite direction, but the shift is also disproportionate to the amount of water in the system. On the other hand, broadening of the signal suggests that involvement of the amine protons in hydrogen bonds stays behind the effect.

Surprisingly, the mutual positions of the NH_2 signals in the three samples changed when NMR measurements were performed after 30 days (Figure 9b). While the chemical shift of the NH_2 protons remained unchanged for the solution that did not contain water, for the solutions containing water the signals of the NH_2 protons changed their positions considerably. In Figure 9b the direction and magnitude of the shift of the NH_2 signal for the samples containing water in relation to the signal for the sample without water are in accordance with the picture of hydrogen bonds formed between amine groups and water molecules.

What is the explanation for the time delay in the NMR spectra of samples containing water? It seems that information both from phase behavior and NMR spectra can throw light on the behavior of the DAB-64 + benzene system at a molecular level on the addition of the small amounts of water. First of all, the fact that the dry DAB-dendr-$(NH_2)_{64}$ easily dissolves in dry benzene suggests that benzene appears to be a good solvent for this macromolecule. In this solution a signal of NH_2 protons involved in intramolecular hydrogen bonds or in interaction with the benzene ring is observed. The position of this signal does not change with time. The upfield shift of the signal with temperature (Figure 5) is in accordance with the decrease of the amount of hydrogen bonds on temperature increase.

When the dendrimer, benzene, and a small amount of water are placed in a vessel, initially two phases are observed. Water is absorbed by the dendrimer lying at the bottom of the vessel. Water present in the system induces clustering of the dendrimer molecules. That is why benzene is not able to dissolve the dendrimer phase as easily as is observed in a system without water. Excessive heating and vigorous mixing are needed for the dissolution of the dendrimer phase. Macroscopic observation indicates that the system is homogeneous. However, the NMR spectra reveal that there are still a lot of dendrimer clusters in the solution within which intermolecular hydrogen bonds between amine groups are formed. The mechanism of water-induced clustering of dendrimer molecules is unknown. In the DAB-64 + benzene saturated with water at 299 K (0.06 wt %

water content) system on average two molecules of water correspond to one dendrimer molecule. Thus, the clustering cannot be explained simply by cross-linking of the dendrimer molecules through water bridges. The NMR spectra measured after 30 days indicate that it takes time for the benzene molecules to break the clusters. Apparently, benzene can compete with NH_2-NH_2 H-bonds, but when water is involved, this interaction is strengthened and temperature and time is need for benzene molecules to destroy the clusters.

Conclusions

The results obtained clearly show that water has a significant and unusual influence on the phase behavior in the DAB−PPI + benzene system. It was proved that even a very small amount of water present in the system induces a liquid−liquid phase split and that removal of water results in complete miscibility of the DAB-64 dendrimer and benzene. These facts suggest that water influences the structure of the dendrimer molecules. The balance between the NH_2, water, and benzene interactions is responsible for the complex phase behavior.

The intriguing water-induced clustering of the DAB-PPI dendrimer molecules raises the question of the difference in the structure of the dendrimer molecules in water-free and water-containing systems. It would be interesting to perform computer simulations and experiments such as dynamic light scattering and viscosity. The more important are consequences of our observations for possible applications. It is apparent that water not only causes clustering of the dendrimer molecules but also changes the adhesive ability of the dendrimer phase toward glass. Application of dendrimers as unimolecular micelles demands that dendrimer molecules exist as single molecules in solution and that they are capable of molecular inclusion. From our study it can be seen that both properties may be ruined by a trace component (in this case water) present in the system.

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Supporting Information Available: ^{1}H NMR spectra of water dissolved in C_6D_6 and MS spectra of the fifth-generation DAB-PPI dendrimer. This material is available free of charge via the Internet at http://pubs.acs.org.

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