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Tri-α-naphthylbenzene as a crystalline or glassy matrix for matrix-assisted laser desorption/ionization: a model system for the study of effects of dispersion of polymer samples at a molecular level[†]

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Tri-α-naphthylbenzene (TαNB) can exist as either a crystalline or glassy solid at ambient temperatures, making it a unique matrix in matrix-assisted laser desorption/ionization (MALDI) spectroscopy. Electrosprayed TαNB is crystalline and has a melting point of $180 \pm 2\,°C$, as measured by differential scanning calorimetry (DSC). A glass of TαNB is obtained upon heating above the crystalline melting point with a glass transition temperature of $68 \pm 2\,°C$ having no remaining crystallinity. MALDI samples containing mass fraction 1% polystyrene (PS) are run in both the crystalline and amorphous states. In the crystalline state, there is a strong spectrum typical of PS, but upon melting and quenching to the glassy state, the MALDI signal disappears. If the transparent, amorphous sample is treated with 1-butanol, it becomes white, and the MALDI signal returns. DSC shows that the 1-butanol treatment leads to the return of some of the crystallinity. Small angle neutron scattering (SANS) shows that the crystalline state has large aggregations of PS while the amorphous state has molecularly dispersed PS molecules. MALDI gives strong signals only when there are large aggregations of polymer molecules, with individually dispersed molecules producing no signal. Published in 2002 by John Wiley & Sons, Ltd.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) of synthetic polymers can yield information about end groups and repeat units, and also quantitative information, such as molecular masses and molecular mass distributions (MMD).[1,2] The function of the matrix is to disperse the polymer and to adsorb optical energy from a laser, ablating the sample into a plume. The ionized polymers are then separated by mass/charge and detected.

The matrix disperses and supports the polymer molecules; therefore, sample preparation is crucial to the success of the technique. The size scale of the dispersion can vary from millimeter heterogeneity that is often visible to the naked eye, to molecular dispersions of individual chains dissolved in the matrix as they would be in a conventional solvent. Additionally, the matrix needs to be non-volatile under the high vacuum conditions of the experiment, so the matrix is most commonly a highly crystalline aromatic molecule. The sample preparation therefore involves a complicated mixture of polymer-matrix-cation-solvent that undergoes simul-

taneous evaporation-crystallization-phase separation that can produce a wide variety of morphologies.

Recent work[3] has used small angle neutron scattering (SANS) to measure the dispersion of polymers in a crystalline matrix. A common MALDI combination, polystyrene (PS) in a dithranol matrix, was found to produce large domains of polymer, containing hundreds or thousands of aggregated chains. Since MALDI matrices are virtually always crystalline, the dispersion size of the polymers in virtually all MALDI measurements is probably quite large. This may not be the optimum morphology for a MALDI measurement, but examples of molecularly dispersed chains in a MALDI matrix are rare.

There are examples of liquid MALDI matrices for biological polymers. Several groups[4–6] have used 3-nitrobenzyl alcohol as a matrix, but it may not be liquid under the conditions of MALDI since this matrix has a melting point around ambient temperature. Also, crystallizable polymers and UV adsorbers are sometimes present so that a single-phase morphology is often difficult to prove.

Previous work on MALDI of synthetic polymers[7] has examined liquid matrices by using mixtures of relatively non-volatile liquids and UV adsorbers. While polymers in the liquid matrices produced MALDI signals, it was found that a higher UV power was required for these matrices than for conventional crystalline matrices and that crystalline matrices had better resolution and could analyze higher

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molecular weight polymers. However, in this previous study, there is no direct proof that there is an absence of crystallinity or phase separation in the liquid mixtures.

In this paper we introduce a new MALDI matrix, tri-α-naphthylbenzene (TαNB), that can form a glassy MALDI matrix at ambient temperatures. In addition, it can be made to crystallize, making it a unique matrix material that can be either crystalline or amorphous, producing morphologies that contain either highly aggregated chains that are strongly phase-separated or individual chains dissolved in the matrix. In this way, we are able to focus our study on the effect of polymer aggregation (from SANS) on the MALDI signal in these two morphological states of the matrix/polymer.

EXPERIMENTAL

Synthesis

TαNB was synthesized by a method derived by Elmorsy *et al.*[8] α-Naphthone (25 g) was dissolved in absolute ethanol (250 mL). The mixture was stirred at ambient temperature and tetrachlorosilane (42 mL) was added under an atmosphere of nitrogen. The mixture turned black and stirring was continued for 20 h. The mixture was extracted with a 3:1 volume mixture of ethanol/methylene chloride. The raw product was recrystallized by dissolving the crude product in methylene chloride, filtering, and adding 95% ethanol until crystals formed. The final yield was 5.5 g (24%) of a light-yellow crystalline solid. All chemicals were obtained from Aldrich Chemical and used as received.*

Sample preparation

A typical solution was made of 0.0007 g of PS or poly(styrene-d_8) (PSD) and 0.03 g TαNB/1 mL THF and were electrosprayed at 5000 V potential with a flow rate of between 4 and 10 μL/min. For neutron scattering samples, the spray was deposited on thin copper windows until a thickness of approximately 0.2 mm was obtained. Similar electrosprayed samples were made on a standard disk for the MALDI experiments. The MALDI samples also contained 0.0009 g of silver trifluoroacetate (AgTFA)/1 mL THF as a cationizing agent. The amounts listed are typically ± 1% of the listed value as is common for such measurements.

UV

Ultraviolet (UV) spectra were taken with a Perkin-Elmer model Lambda spectrometer in the two-beam mode at 10 nm min^{-1} with a 0.1-mm slit width. Samples were made of TαNB in methylene chloride at concentrations of approximately mass fraction 1% and the solution was evaporated on a quartz plate producing a translucent film. After the UV spectrum was taken, the plate was placed in an oven at 200 °C for 2 min, producing a transparent film and the UV spectrum was remeasured. The uncertainties are not plotted

in the figures since the results of relative adsorbance are qualitative only.

DSC

Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC 7 calorimeter from 30 to 200 °C at 10 °C min^{-1} under a nitrogen atmosphere. After the maximum temperature was reached the sample was cooled at 10 °C min^{-1} to 30 °C and the run was repeated. In a second set of experiments, TαNB in THF was electrosprayed onto a copper substrate and scraped into a DSC pan. The sample was scanned twice as before, but, after the second scan, the pan was pried open and a drop of 1-butanol was placed on the TαNB. The solvent was allowed to evaporate and the pan was placed in vacuum at ambient temperature to remove all of the 1-butanol before the pan was resealed and the DSC run was repeated. The uncertainties are not plotted in the figures since the results of relative heat flow are qualitative only.

SANS

SANS experiments were carried out on the 8m (NG1) instrument of the National Institute of Standards Technology Cold Neutron Research Facility in Gaithersburg, MD, USA.[9] The wavelength, λ, of the incident beam was 12 Å. The observed scattering intensity at a given temperature is collected over a two-dimensional detector and corrected for empty cell, background radiation and detector inhomogeneity. It is then normalized against H$_2$O, which serves as a secondary standard, to give the absolute intensity. Finally, it is circularly averaged to give the q dependence of the coherent scattering cross-section, $I(q) = d\Sigma/d\Omega(q)$, in absolute units (cm^{-1}). The uncertainties are calculated as the estimated standard deviation of the mean and the total combined uncertainty is not given as comparisons are made with data obtained under the same conditions. In cases where the limits are smaller than the plotted symbols, the limits are left out for clarity. Fits of the scattering data are made by a least-squares fit of the data giving an average and a standard deviation to the fit. All temperatures reported are within ± 1 °C as determined by previous experience with the controller.

PSD (Polymer Source) and TαNB in THF was electrosprayed onto a copper disk for the SANS experiment along with a pure TαNB blank. The copper provided an electrically conductive surface necessary for the electrospray process and had a high transmission of neutrons for the SANS measurement. The sample had a smooth surface, without any significant roughness to produce surface scattering. The TαNB blank had weak coherent scattering due to the polycrystalline-amorphous morphology, but the relative intensity was negligible compared to the scattering of the mass fraction 1% PSD electrosprayed sample.

Two amorphous samples were made: mass fraction 1% PSD 188 000 g mol^{-1} was purchased from Polymer Laboratories in TαNB and a pure TαNB blank was prepared by evaporation of methylene chloride solutions to dryness and placing the solids into quartz liquid cells. They were annealed at 200 °C to melt the crystals and cooled to room temperature.
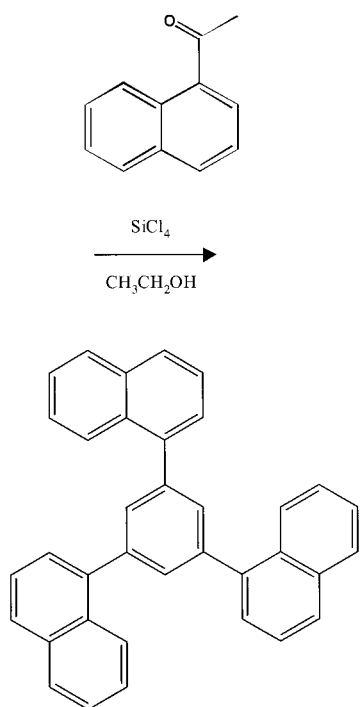
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* Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation by the National Institute of Standards and Technology nor does it imply that the material or equipment identified is necessarily the best available for this purpose.

Figure 1. Synthesis and structure of tri-α-naphthylbenzene (TαNB).

MALDI

TαNB was used as the matrix, tetrahydrofuran (THF) was used as solvent and silver trifluoroacetate (AgTFA) was used as a cationizing agent in all experiments. PSD of molecular mass $3900\,g\,mol^{-1}$ was purchased from Polymer Source. The matrix, PSD, and AgTFA solutions were prepared separately and mixed prior to deposition on the sample target by electrospray. Experiments were performed on a Bruker Reflex II MALDI-TOF mass spectrometer operated in reflectron mode using delayed extraction, with a dual microchannel plate detector and a 3-ns pulse width nitrogen laser.

The first MALDI experiments were performed on the electrosprayed sample plate shortly after deposition. The sample plate was placed in an oven at 200 °C for 5 min until the film became transparent and the second set of MALDI experiments were run. The annealed sample was modified by placing a drop of 1-butanol on the film and allowing it to evaporate and the third set of MALDI experiments were performed. The uncertainties are not plotted in the figures since the results of relative detector response are qualitative only.

RESULTS AND DISCUSSION

Synthesis

TαNB can be easily synthesized in quantities of several grams in a single step by the trimerization of α-naphthone by the procedure of Elmorsy *et al.*[8] Recrystallization gives a light-yellow crystalline material that has suitable optical, thermal, and solubility characteristics for use as a model MALDI matrix. Figure 1 shows the synthesis and final structure of TαNB.

UV

Figure 2 shows the UV adsorbance of solid TαNB in the crystalline and glassy states. The 'adsorbance' of the crystalline sample is higher than the glassy sample, but this is likely due to scattering of the UV beam by the microscopic
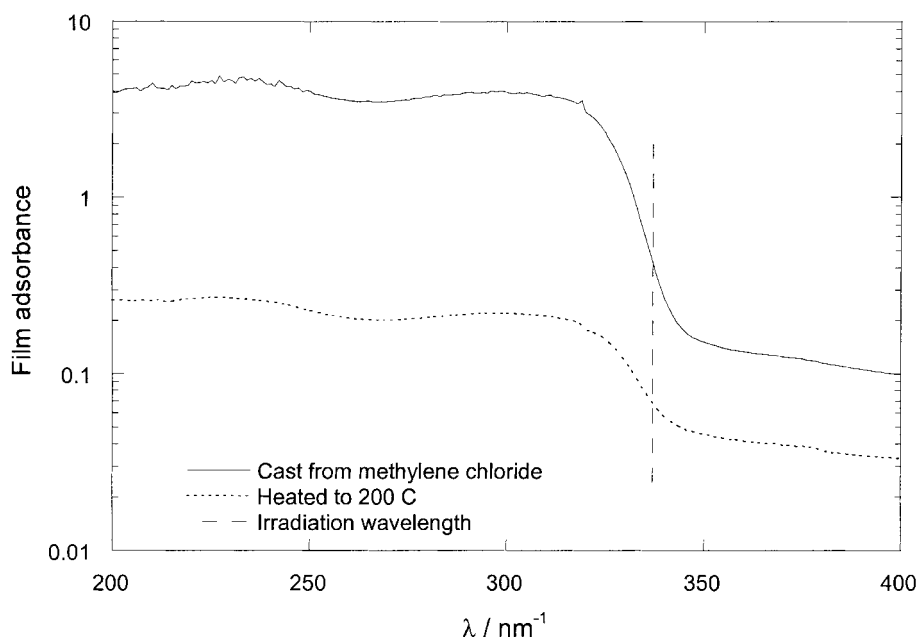

Figure 2. UV adsorbance of TαNB film as cast from methylene chloride (crystalline) and after heating to 200 °C (glassy and amorphous). The vertical line is the MALDI laser wavelength of $337\,nm^{-1}$. The amount of light adsorbed and scattered in the crystalline state is five times greater than the amount adsorbed in the glassy state.
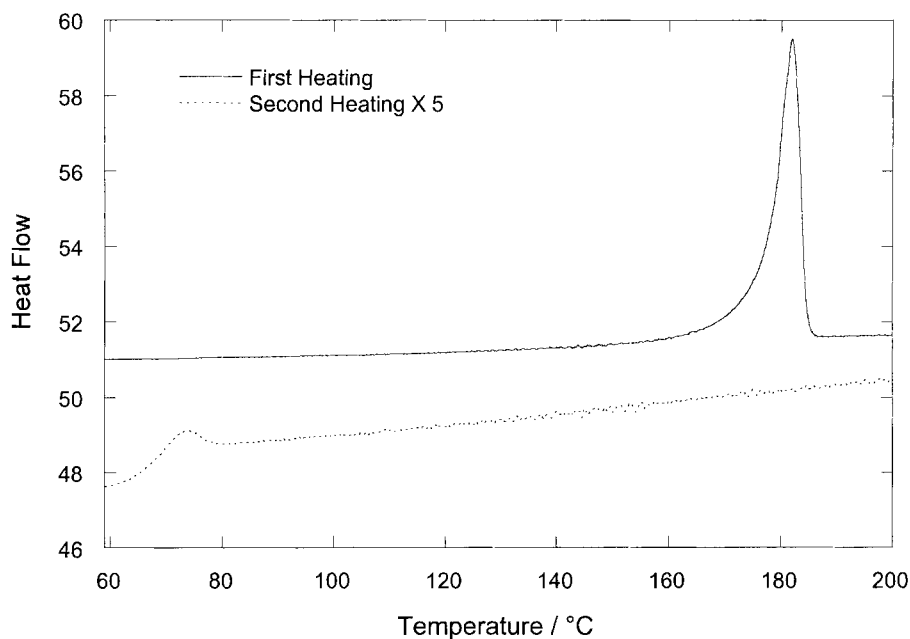
Figure 3. DSC of TαNB. The first heating shows the crystalline melting point with the peak at 180 ± 2 °C. On the second heating, after annealing, the crystalline melting peak disappears and a glass transition appears at 68 ± 2 °C.

structure of the crystals and not due to adsorbance of the beam by the sample. If the incident beam is scattered out of the sample and not adsorbed, then it does not cause heating and ablation of the polymer and matrix. There is a rapid drop-off in the adsorbance at wavelengths higher than the peak position with the value at the laser wavelength being only 11% of the adsorbance at the peak. Even though the adsorbance characteristics are not optimized for the laser used, efficient MALDI is still obtained for this matrix.

DSC

Figure 3 shows the thermal characteristics of TαNB. The sample was prepared by the evaporation of a methylene chloride solution that left a powder that appeared to be comprised of crystals. The first heating shows the crystalline melting point with the peak at 180 ± 2 °C and no sign of other thermal events at other temperatures. On the second heating, a T_g appears at 68 ± 2 °C and the crystalline melting peak disappears. The sample was annealed at 150 °C for 8 h and the scan was repeated. The results were the same as after the first melting, without reappearance of the crystalline melting peak.

TαNB was electrosprayed from methylene chloride and scraped off of the substrate into a DSC pan. The scan shows the crystalline melting on the first run with a complete disappearance upon the second scan (see Fig. 4). Upon treatment with 1-butanol, a weak crystalline melting peak reappears. Therefore, the crystalline state of the sample can be easily controlled by sample preparation and treatment, with a highly crystalline sample being initially created by electrospray that can be converted to a glassy sample by annealing above the melting point for a few minutes. The 1-butanol treatment causes some of the crystallinity to return by dissolving the amorphous TαNB which crystallizes upon solvent evaporation.

SANS

Scattering techniques provide information on the organization of material in the scattering volume. Different materials have scattering contrast factors that depend on the atomic content of the materials and the type of scattered radiation. SANS contrast factors can be modified by substitution of deuterium for hydrogen in one of the components. The electrosprayed MALDI samples have contributions to SANS from the relative sizes of the matrix and polymer components. To make the contrast between the components as high as possible, a polymer is used with deuterium substitution for the hydrogen normally found in the polymer. Therefore, a matrix that contains hydrogen provides strong SANS contrast and produces a strong SANS signal. The contrast provided by deuterium substitution dominates the scattering, making contributions from (amorphous-crystalline) contrast negligible.

SANS can probe size scales in the range 10–1000 Å and determine the organization of the chains. A solution of molecularly dispersed polymers in concentrations typical of MALDI samples has scattering of Ornstein-Zernike[10] type

$$I(q) = I(q = 0)/(1 + \xi_{OZ}^2 q^2) \tag{1}$$

where $I(q)$ is the scattered intensity at scattering vector $q = 4\pi sin(\theta/2)/\lambda$ with θ being the scattered angle and λ the neutron wavelength and ξ_{OZ} is a characteristic size related to the radius-of-gyration as $R_g = 3^{1/2} \xi_{OZ}$. At higher values of q, there is a characteristic power law of

$$I(q) \propto q^{-2} \tag{2}$$

If the polymer is phase-separated into two phases with a random distribution of phase sizes,[11] the scattering follows Eqn. (3).
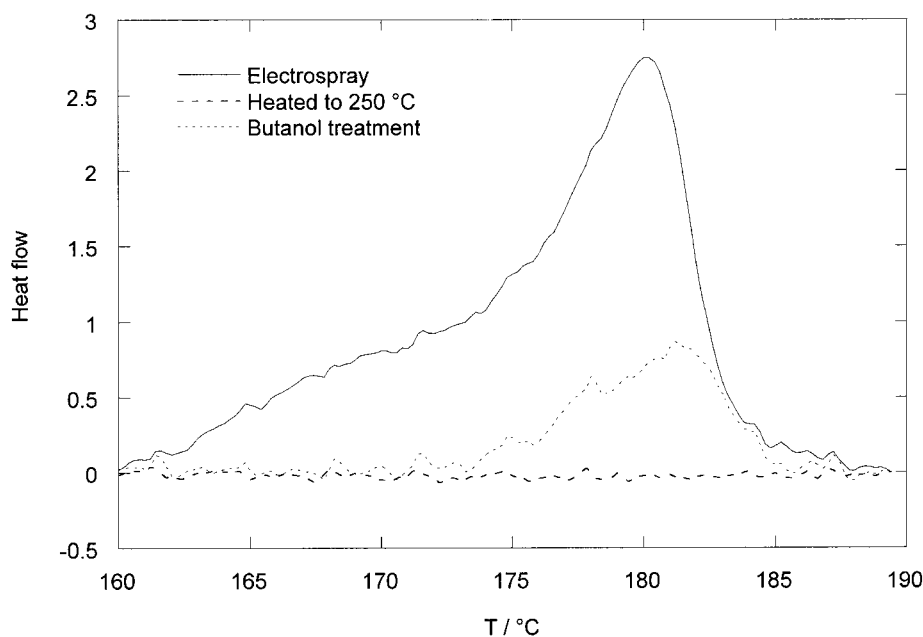
$$I(q) = I(q = 0)/(1 + \xi_D^2 q^2)^2 \tag{3}$$

Figure 4. DSC of electrosprayed TαNB. The first heating shows the crystalline melting point with the peak at $180 \pm 2\,°C$. On the second heating, the crystalline melting peak disappears. The TαNB is covered with 1-butanol and allowed to evaporate slowly. The crystalline melting peak returns upon reheating.

In this equation ξ_D is a characteristic Debye correlation length and is related to the average chord length of the polymer phase as $\xi_{PSD} = \xi_D/(1 - \phi_{PSD})$ where ϕ_{PSD} is the volume fraction of polymer in the mixture. The function form of this equation is similar to that of Eqn. (1) except that the denominator is squared producing a distinctive high q form. At higher values of q, there is a characteristic power law of

$$I(q) \propto q^{-4} \qquad (4)$$

Therefore, the limiting power law can distinguish the morphological type, dissolved linear chains giving -2 or phase-separated collections of many chains giving -4. Fits of the appropriate equation gives a radius-of-gyration of the dissolved PSD or the average size of the phase-separated PSD, respectively.

Figure 5 shows the SANS of the electrosprayed and the melted PSD/TαNB samples. The high q slopes of the two morphologies are -4 for the electrosprayed and -2 for the melted samples signifying strongly phase-separated and dissolved PSD, respectively. Fits of Eqns (1) and (3) give an $R_g = 134 \pm 13\,Å$ for the glassy $188\,000\,g\,mol^{-1}$ PSD and $\xi_{PSD} = 1050 \pm 105\,Å$ for the crystalline electrosprayed $27\,000\,g\,mol^{-1}$ PSD. Two different molecular masses were used in this example to emphasize the thermodynamic effects. Due to entropic effects, lower molecular masses are more likely to dissolve than high ones, so high and low molecular masses were chosen for the melted and electrospray examples given here.

Therefore, electrosprayed samples are strongly phase-separated into large domains while the samples that were annealed above the crystalline melting point and quenched below T_g formed solid solutions. The DSC results have

shown that the electrosprayed samples are crystalline, while the annealed samples are amorphous. When TαNB is crystallized from solution, the crystallization process forces the polymer out of the crystallites into a separate phase; however, when molten TαNB forms a glass upon cooling, the polymer remains dispersed in the matrix. Therefore, TαNB is an ideal model matrix for the study of the effect of polymer dispersion on the efficiency of MALDI.

MALDI

The original electrosprayed sample produced an opaque film characteristic of a phase-separated solid-solid mixture. This is consistent with the SANS results of an electrosprayed sample of a similar mixture. It gave a strong MALDI signal typical of polystyrene samples in conventional matrices such as dithranol, indole acrylic acid, or all-trans-retinoic acid. An electrosprayed TαNB-PSD result is shown in Fig. 6 as the uppermost plot showing the complete separation of the styrene repeat units.

Upon annealing, the film became optically transparent, suggesting that a miscible mixture had been formed. This is consistent with the SANS results of an annealed sample of a similar mixture. MALDI was performed on the same physical sample that gave a strong signal before annealing, but after annealing the signal diminishes to near baseline noise. The middle plot of Fig. 6 shows the signal of the annealed sample for the same number of laser pulses.

The near complete loss of signal could possibly be due to irreparable changes in the sample due to the heat treatment such as loss of the cationizing agent or the degradation of the polystyrene. One way to answer this question is to treat the annealed sample in a way that would regain the original morphology. DSC and SANS have shown that the major
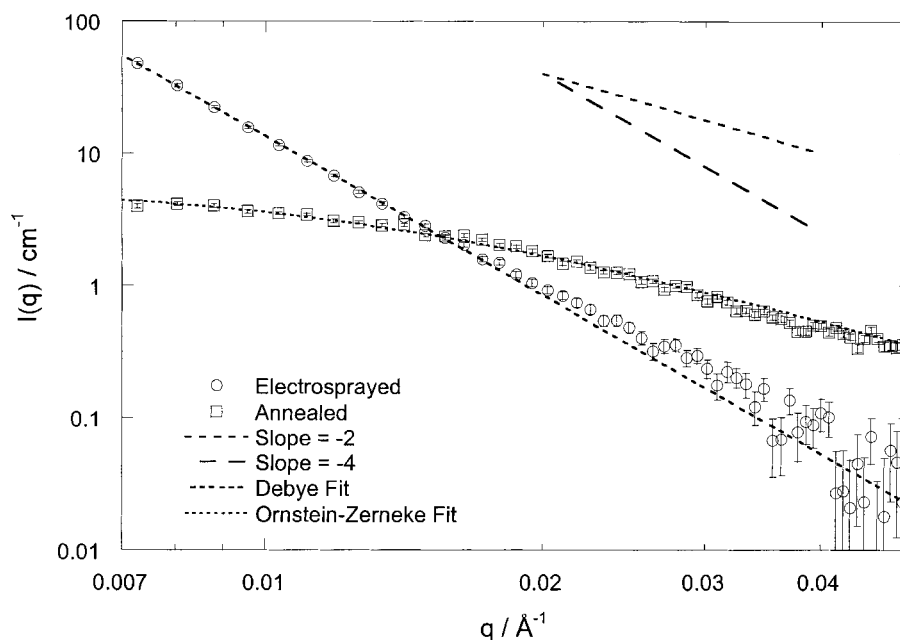
Figure 5. SANS of the electrosprayed and the melted PSD/TαNB samples. The high q slopes of the two morphologies are -4 for the electrosprayed and -2 for the melted samples signifying strongly phase-separated and dissolved PSD, respectively. $Rg = 134 \pm 13$ Å for the melted 188000 g mol^{-1} PSD and $\xi_{PSD} = 1050 \pm 105$ Å for the electrosprayed 27000 g mol^{-1} PSD.

changes from the melting process are loss of crystallinity and loss of the phase-separated morphology. In principle, the crystallinity could be regained by annealing the sample at a temperature below the crystalline melting point of TαNB, but the required time would have been excessive.

The samples were modified by placing a drop of 1-butanol on the surface of the film. 1-Butanol is a good solvent for TαNB but a non-solvent for polystyrene. Upon evaporation of the solvent, the film had become opaque, suggesting that the two-phase morphology had been regained. MALDI of this sample produced a strong signal, comparable to the original electrosprayed sample. The lower plot of Fig. 6 shows the signal. The molecular mass of the electrosprayed sample is $M_n = 3970 \pm 150$ g mol^{-1} with the uncertainties being one standard deviation calculated from the statistics of the fit. After heating and 1-butanol treatment the molecular mass is $M_n = 3770 \pm 300$ g mol^{-1} so the heating and solvent treatment do not affect the molecular mass distribution.

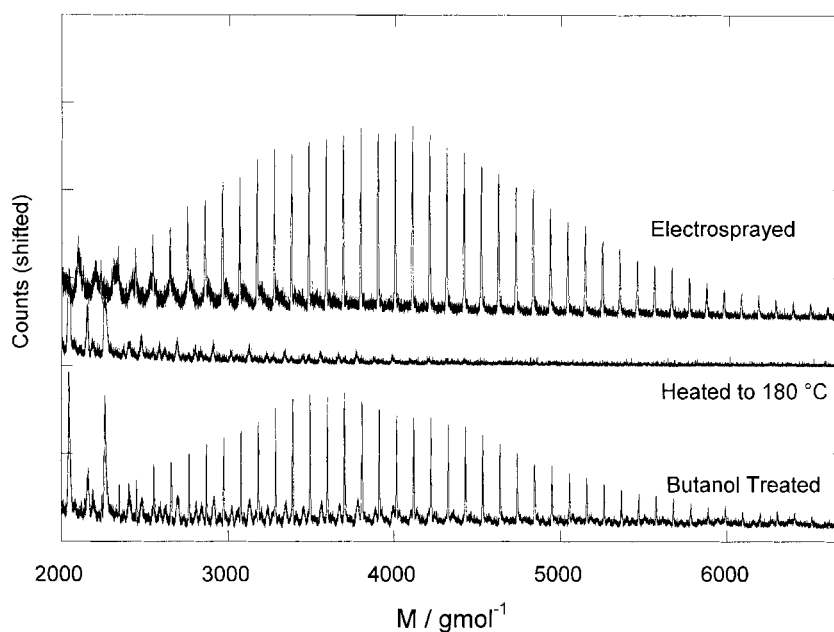**Figure 6.** MALDI of PS in TαNB. Upper curve, as electrosprayed; middle curve, heated to 190 °C; lower curve, treated with 1-butanol.

Therefore, the disappearance of the MALDI signal with melting of the TαNB matrix is not due to an irreversible change in the sample. Rather, it seems likely that polymer chains that are molecularly dispersed in a matrix produce far less signal than ones grouped in large domains separated by crystallites. Previous work[7] that examined liquid matrices found that they produced MALDI signals; however, a higher UV power was required for these matrices than for conventional crystalline matrices and the crystalline matrices had better resolution and could analyze higher molecular weight polymers. However, these studies reported no direct comparison between matrices that could be either crystalline or amorphous, as is true for TαNB.

CONCLUSIONS

TαNB has been examined as a matrix in MALDI spectrometry. It has the unique ability among MALDI matrices to be either crystalline or an amorphous glass. Electrospray sample preparation initially produces the crystalline state, but heating the sample above the melting point causes the crystallinity to disappear and forms a material that is a glass at ambient temperatures. SANS shows that PS is aggregated into large domains initially, and that upon melting, the PS dissolves in the glassy TαNB, breaking up the aggregates and causing a molecular dispersion of the chains.

MALDI of PS samples in the crystalline TαNB gives results similar to conventional crystalline matrices such as dithranol. In the glassy state in which the chains are molecularly dispersed, MALDI no longer produces usable spectra under conditions that gave strong spectra previously. When the sample is then treated with 1-butanol, a strong MALDI spectrum returns. Thus, it seems that, to obtain a strong MALDI signal for PS, molecular dispersion is disadvantageous and the presence of large domains of polymer interspersed with large domains of the matrix increases the relative signal strength.

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