Electronic Supplementary Material (ESI) for Analytical Methods

Towards the rationalization of the MALDI process: a combined mass spectrometry/solid-state NMR approach

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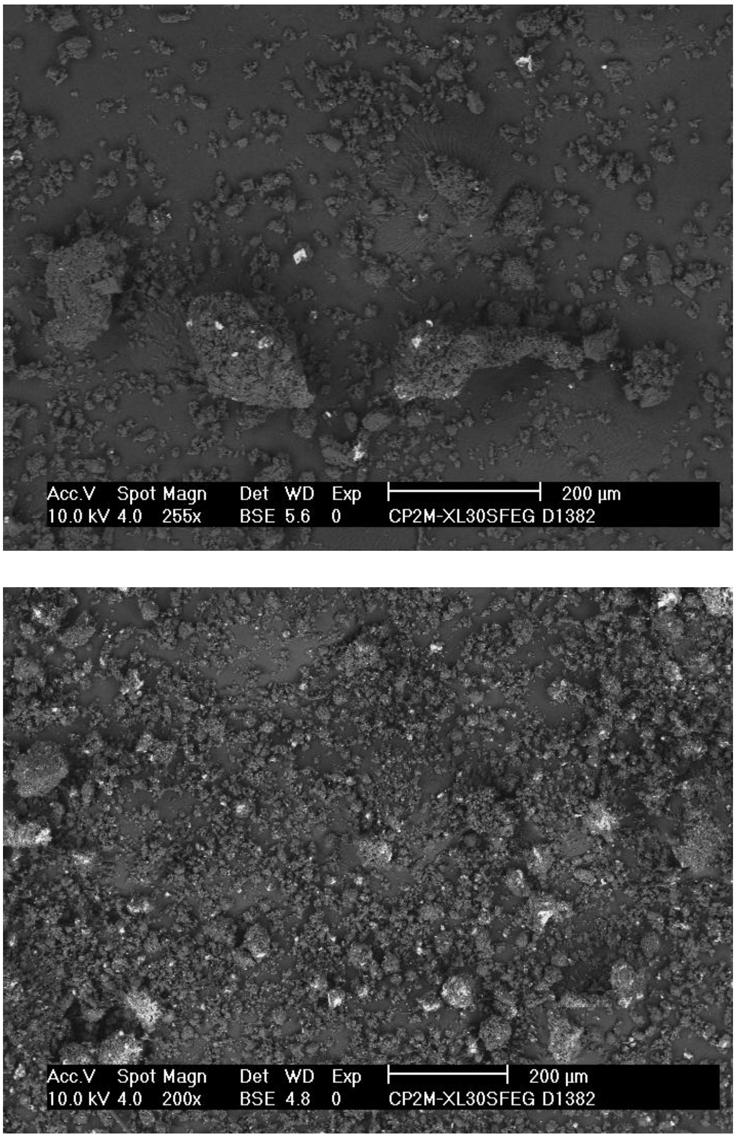
SUPPORTING INFORMATION



Supplemental Figure S-1. SEM images of solvent-free MALDI samples obtained after grinding the ternary solid mixture during 2 min (upper side) or 16 min (lower side).

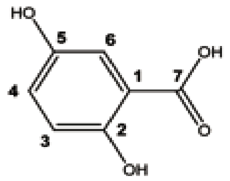
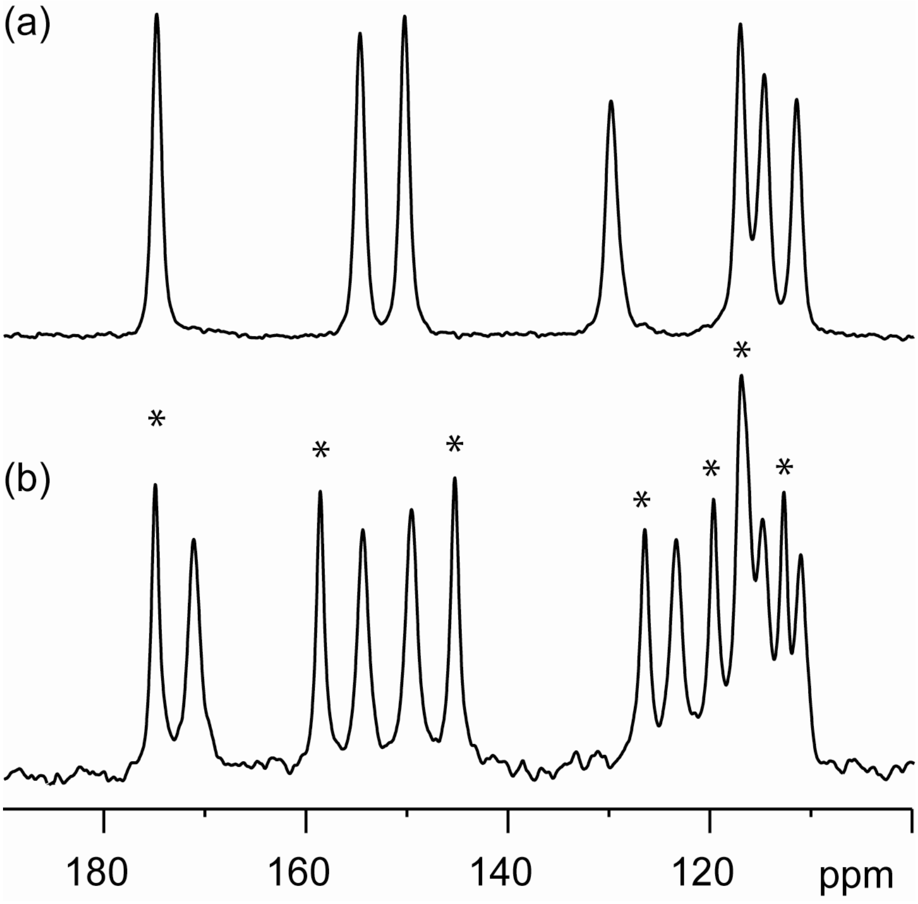


Figure		C1	C2	C3	C4	C5	C6	C7
2a	Phase I	**1**: 112.9	**2**: 158.7	**3**: 119.9	**4**: 126.6	**5**: 145.4	**6**: 117.3	**7**: 175.0
	Phase II	-	-	-	-	-	-	-
	Phase III	-	-	-	-	-	-	-
2b	Phase I	**1**: 112.9	**2**: 158.7	**3**: 119.9	**4**: 126.6	**5**: 145.4	**6**: 117.3	**7**: 175.0
	Phase II	**1'**:111.7	**2'**:154.8	**3'**:117.3	**4'**: 130.0	**5'**: 150.4	**6'**: 114.8	-
	Phase III	ND	ND	ND	ND	ND	ND	ND
2c	Phase I	**1**: 112.9	**2**: 158.7	**3**: 119.9	**4**: 126.6	**5**: 145.4	**6**: 117.3	**7**: 175.0
	Phase II	**1'**:111.7	**2'**:154.8	**3'**:117.3	**4'**: 130.0	**5'**: 150.4	**6'**: 114.8	-
	Phase III	-	-	-	**4"**: 123.5	-	-	**7"**: 171.1

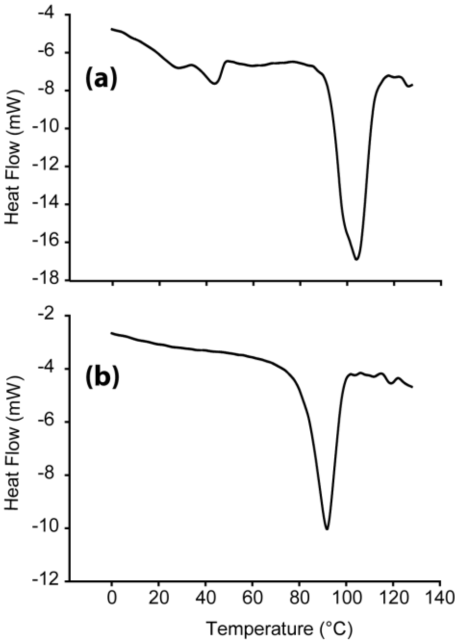
Supplemental Table S-1. Chemical shift assignments (in ppm) of ^{13}C NMR spectra of Figure 2. Figure 2a: ^{13}C CP/MAS NMR spectrum of pure 2,5-DHB; Figure 2b: ^{13}C CP/MAS NMR spectrum of the solvent-free MALDI sample (2,5-DHB/PEG/CsCl, 50:1:10) ground for 2 min; Figure 2c: ^{13}C CP/MAS NMR spectrum of the solvent-free MALDI sample (2,5-DHB/PEG/CsCl, 50:1:10) ground for 16 min. Numbering refers to the structure of 2,5-DHB shown below. Unprimed, primed, and double-primed numbers (in bold) refer to carbon signals of 2,5-DHB belonging to "phase I", "phase II" and "phase III", respectively (see text).



Supplemental Figure S-2. ^{13}C CP/MAS spectra of a) 2,5-DHB:PEG (20:1) and b) 2,5-DHB:CsCl (1:1) binary mixtures ground during 16 min under ambient atmosphere conditions. a) corresponds to the ^{13}C spectrum of 2,5-DHB molecules belonging to "phase II" (2,5-DHB/PEG co-crystal), while b) shows the presence of 2,5-DHB both as "phase III" (2,5-DHB/CsCl co-crystal) and "phase I" (pure 2,5-DHB), signals ascribable to the latter being annotated with asterisks.

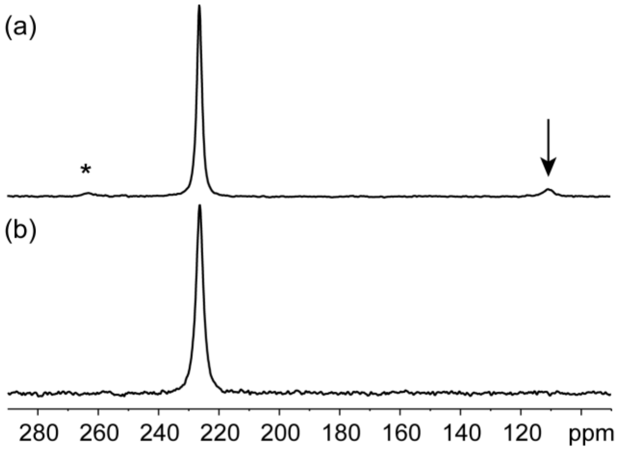
Differential scanning calorimetry

Complementary information about formation of "phase II" could be obtained by differential scanning calorimetry (DSC). A melting peak at 44°C in the 2M sample thermogram (Supplemental Figure S-3a) revealed the presence of residual crystalline PEG, and was also observed for pure PEG ground in the same conditions. Its amount was determined to be 10% (w/w), which is about the detection limit for this species in NMR.



Supplemental Figure S-3. Differential scanning calorimetry thermograms of PEG in the ternary mixture ground for a) 2 min and b) 16 min. DSC measurements were performed on a DSC 2920 from TA Instruments (New Castle, DE). Samples were sealed in aluminum pan and heated at a rate of 5°C/min from -60 to 130°C under nitrogen atmosphere.

The presence of a second melting point in both 2M and 16M samples (Supplemental Figures S-3a and S-3b, respectively) indicates that a crystalline complex has formed between the polymer and a second compound.[1, 2] The temperature range of this additional signal (between 90 and 100°C) was reported to be characteristic of a complex between PEG and hydroxybenzene compounds,[1] and is thus consistent with the proposed formation of "phase II" composed of PEG and 2,5-DHB in the studied solvent-free MALDI samples. In support of this, the melting temperature of this peak is clearly distinct from that reported for pure 2,5-DHB (~ 205°C) and intermediate between that of pure PEG and pure 2,5 DHB (CsCl melts at a much higher temperature, *i.e.*, 645°C). In the DSC thermogram obtained for the 16M sample (Supplemental Figure S-3b), the 44°C melting point diagnostic of the pure PEG crystalline phase is no longer observed and the second melting point is measured at a slightly lower temperature as compared to 2M. A longer grinding time was shown to improve homogeneity of the sample and of the solid particle size (as shown in Supplemental Figure S-1), hence accounting for the slightly lower melting point detected in 16M as compared to 2M. This assumption was further supported by the width of this melting point signal, which decreased as the grinding time increased.



Supplemental Figure S-4. ^{133}Cs DE/MAS spectra of solvent-free MALDI samples ground for 16 min under a) ambient atmosphere and b) nitrogen atmosphere. The peak indicated with an arrow in a) corresponds to Cs atoms in “phase III”. Asterisks denote spinning sidebands.

Sample homogeneity. Useful information about the distribution of the different matrix phases in solvent-free MALDI samples as the grinding time is increased could be drawn from longitudinal ^{1}H relaxation times, T_1. ^{1}H spin-lattice relaxation times in the laboratory frame (T_1) were measured using a ^{13}C-detected saturation recovery experiment.[3] Spectral deconvolution of the so-obtained ^{13}C NMR spectra was performed using the software DMFIT.[4] Non-linear fit of the experimental ^{1}H T_1 recovery curves was performed using the software Mathematica (v. 8.0, Wolfram Research Inc., USA).

In all cases, the experimental ^{1}H T_1 relaxation curves could be described by a single exponential function, indicating that each observed ^{13}C signal is diagnostic of a single phase that is homogeneous on a ~100 Å scale. ^{1}H T_1 of the crystalline matrix ("phase I", indicated as T_1^{I}), either pure or in the MALDI samples, was shown to decrease upon increasing grinding times, from more than 800 s for pure, not ground, 2,5-DHB, down to 110 s and 29 s for 2M and 16M samples, respectively. This could be either due to the decrease of the 2,5-DHB particle size and the associated enhanced matrix surface area, or to an increased homogeneity of the solid sample, in which each domain composed of a given matrix phase becomes smaller. Shorter relaxation times were measured for signals assigned to 2,5-DHB in interaction with PEG chains (matrix "phase II"). For 2,5-DHB, T_1^{II} was found to be 7.1 ± 0.9 s in the 2M sample and 7.3 ± 0.8 s in the 16M sample. PEG longitudinal ^{1}H relaxation times were 6.6 ± 0.4 s and 7.3 ± 0.4 s in 2M and 16M samples, respectively, in contrast to the 2.5 ± 0.2 s value measured in both the crystalline and amorphous components of pure PEG. The ^{1}H T_1 values measured for 2,5-DHB and PEG in each sample are almost equal and intermediate between the values of the two pure components, indicating a complete average of the relaxation times operated by spin diffusion. This confirms that a very intimate mixing occurred between 2,5-DHB and PEG in the two ground samples, which is consistent with the formation of "phase II" in both 2M and 16M. In addition, the average linear dimensions of the so-formed "phase II" homogeneous domains were estimated to be about 500 Å by taking into account a simplified model for the diffusion (see below).[5] In both 2M and 16M, T_1^{I} was about one order of magnitude larger than T_1^{II}, clearly demonstrating that matrix molecules in the crystalline phase and those in interaction with PEG belong to two distinct phases (i.e., they are not intimately mixed on a spatial range of 500 Å). The intensity of peaks assigned to 2,5-DHB in interaction with Cs in "phase III" (annotated by black arrows in Figure 2b) was too weak to allow the measurement of ^{1}H T_1^{III}. However, relative intensity of these signals with respect to those of "phase II" was observed to increase in experiments where the recycle delay was increased, indicating that T_1^{III} is larger than T_1^{II} and hence that "phase III" forms domains larger than 500 Å and morphologically distinct from "phase II" (and "phase I").

Spin Diffusion. Spin diffusion is a process involving the spatial transfer of magnetization without diffusion of matter, and is mostly effective in abundant spin systems, like protons. This phenomenon acts on the spin system by averaging out all the spatial gradients of longitudinal magnetization *via* the agency of the dipolar coupling between homonuclear spins. Spatial gradients of magnetization correspond to region of the sample characterized by a different proton spin-lattice relaxation time, T_1. Therefore, measurement of such relaxation times is an indication of how much the spin diffusion process proceeds, which is related to the morphology of the sample itself. The average diffusion path length of the magnetization can be estimated using the following equation:

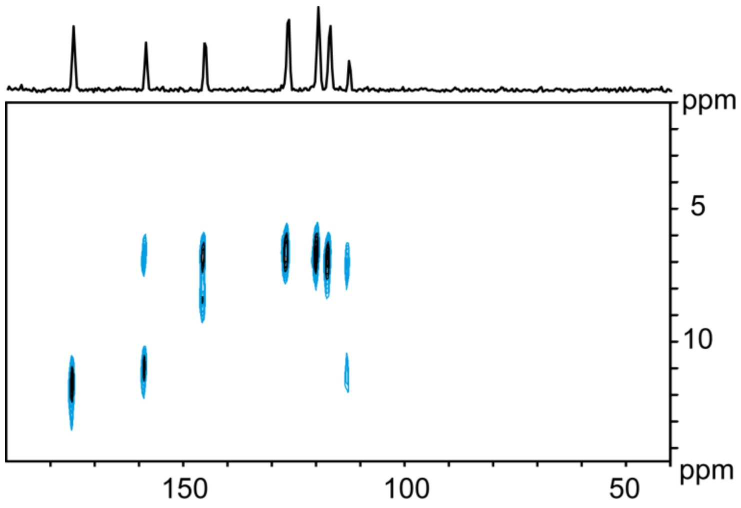
$$\langle r^2 \rangle = n \times D \times \tau$$

where *n* is a numeric factor going from 1 to 6 depending on the geometrical model chosen to describe the diffusion, *D* is the diffusion coefficient and τ indicates the time left for diffusion to proceed (*i.e.*, the measured relaxation time). This equation shows that it is possible to obtain information about the domain dimensions in heterogeneous materials from the knowledge of the ^{1}H relaxation time of a (homogeneous) domain: smaller domains need shorter times to re-equilibrate the magnetization, while this time becomes longer for larger domains. In the present analysis, *n* was fixed to to 6, to take into account a tridimensional diffusion; a value of 6 x 10^{-16} m^2/s was chosen for the coefficient *D*, which is typical of solid polymers.

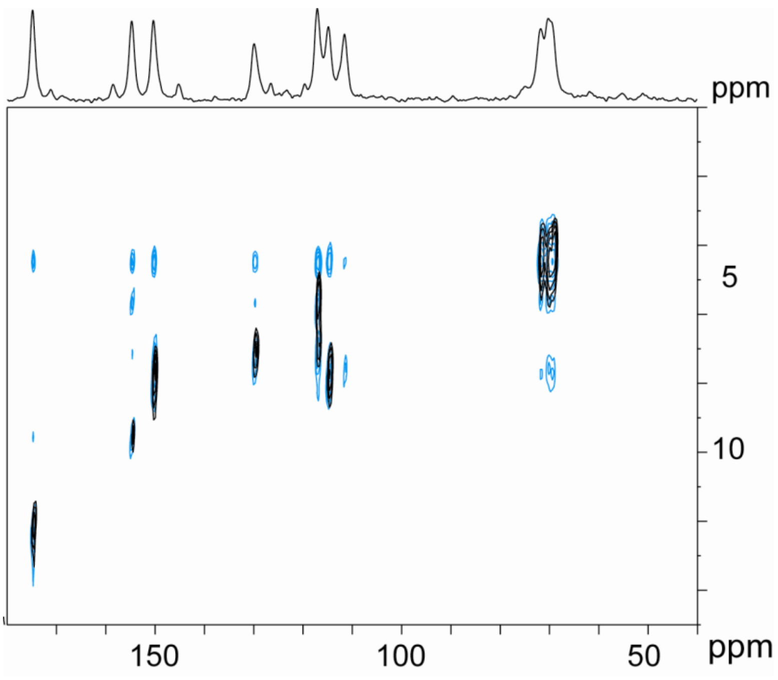
2D heteronuclear correlation (HETCOR) experiments

Atom #	Pure 2,5-DHB (matrix “phase I”)		2,5-DHB/PEG (matrix “phase II”)		2,5-DHB/CsCl (matrix “phase III”)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	112.9		111.7		111.2	
2	158.7		154.8		154.5	
3	119.9	6.6	117.3	5.4	116.6	6.1
4	126.6	6.6	130.0	7.0	123.5	7.2
5	145.4		150.4		149.7	
6	117.3	6.9	114.8	7.6	114.9	6.9
7	175.0		174.9		171.1	
$\mathrm{O\underline{H}2}$		11.0		9.4		10.7
$\mathrm{O\underline{H}5}$		8.4		7.6		7.5
$\mathrm{O\underline{H}7}$		11.8		12.2		12.2

Supplemental Table S-2. Chemical shift assignments (in ppm) of ^{13}C and ^{1}H nuclei of 2,5-DHB in ^{1}H-^{13}C HETCOR 2D experiments.



Supplemental Figure S-5. ^{1}H-^{13}C HETCOR spectra of pure 2,5-DHB, acquired spinning the sample at 10 kHz. Black and blue correlation peaks correspond to short and long-range ^{1}H-^{13}C dipolar interactions, respectively.



Supplemental Figure S-6. ^{1}H-^{13}C HETCOR spectrum of the 2,5-DHB/PEG/CsCl mixture ground for 16 min (16M). Black and blue cross-peaks indicate short and long-range ^{1}H-^{13}C dipolar interactions, respectively.

References

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