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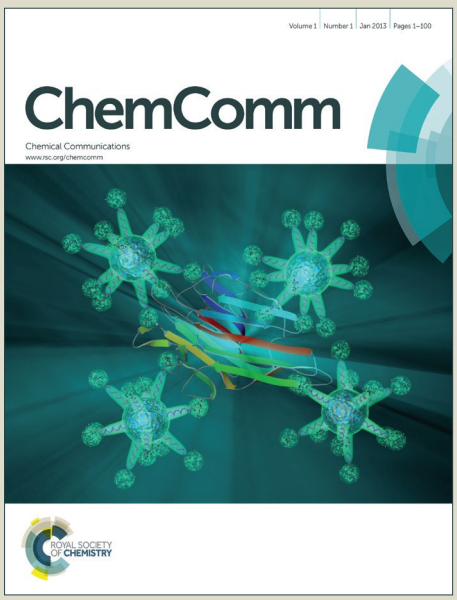

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Dynamic Nuclear Polarization of a glassy matrix prepared by solid state mechanochemical amorphization of crystalline substances

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A mechanochemical "solvent-free" route is presented for the preparation of solid samples ready to be employed in the Dynamic Nuclear Polarization (DNP). ^{1}H-DNP build-up curves at 3.46 T as a function of temperature and radical concentration show steady state nuclear polarization of 10% (0.5% TEMPO concentration at 1.75 K).

The extraordinary potential of Nuclear Magnetic Resonance (NMR) in providing manifold microscopic information about living and non-living matter[1] is somehow constrained by the technique's intrinsic low sensitivity. At room temperature and for a standard laboratory magnetic field (1÷10 T) only one nuclear spin over 10^5 – 10^6 effectively contributes to the NMR signal. This is due to the small energy separation between different Zeeman levels with respect to the available thermal energy, that leads to almost equally populated states. Under given circumstances, hyperpolarization techniques (Golman 2003), such as "Brute Force", Optical Pumping, Para-Hydrogen Induced Polarization and Dynamic Nuclear Polarization (DNP), have been shown to be able to overcome the sensitivity limitations of NMR, thus opening up unexpected experimental possibilities.[2] Indeed, solid state DNP allows the nuclear magnetization to be enhanced by a factor > 10,000[3] and, therefore, when combined with final rapid dissolution of the target sample (DNP-dissolution), can be fruitfully used for tracking metabolic events in vivo.[4]

In solid state DNP, nuclei are hyperpolarized in a magnetic field of a few Tesla by low temperature (usually below 4 K) microwave irradiation of the target sample doped with a radical. Typically, the target sample is a metabolic substrate such as pyruvate or fumarate and the radical belongs to the Trityl or to the TEMPO family. In order for the DNP process to be effective, the nuclei of the target sample and the unpaired electrons of the stable radical species need to be suitably dispersed so that an efficient hyperfine coupling occurs in the mixed solid solution.[5] This implies an amorphous state that is usually obtained by flash freezing the parent liquid phase. However, when the target sample is in a solid crystalline form at room temperature, either a melting procedure or dissolution in a glass-forming agent (such as glycerol or DMSO) must be applied before the freezing step.[6] Both procedures have several drawbacks. Melting is an additional step that must be performed on site, inhibiting any industrial and pharmaceutical production of a "ready to use" formulation. In particular, both melting and the subsequent cooling need to be carefully monitored to ensure the absence of sample degradation during the complete melting and of recrystallization during the rapid cooling step. This means that both the target and the radical must have a common window of melting and stability, which is often not the case. The dissolution procedure implies the addition of a glass-forming agent that may introduce toxicological issues and metabolic interferences in the event of the product being intended for medical use. Moreover, the presence of a sort of "dead volume" in the sample to be polarized gives rise to a suboptimal employment of the whole DNP set up: the larger the sample volume, the less efficient the cooling, the microwave irradiation and the final DNP-dissolution.

In this work, a novel mechanochemical procedure is exploited in order to obtain a solid amorphous sample, ready for use in DNP, starting from compounds which are crystalline at room temperature. This "solvent-free" approach overcomes the aforementioned drawbacks characterizing the preparation methods known in the literature. In particular, it shows that it is possible to evenly spread a radical molecule into a solid substrate target by co-milling the two species at room temperature, without the use of vitrifying liquid media. Here the effectiveness of this procedure is demonstrated by determining the amorphization and polarization characteristics of ad-hoc prepared samples, based on trehalose, a well-known sugar

deeply investigated both in crystalline and glassy state.[7] In particular, trehalose is able to form glass solutions with several other molecular materials by co-milling (driven molecular alloys)[8] and is taken as representative of non-ionic molecules that undergo rather fast dissolution in water.

Thus, several samples with different concentrations of TEMPO in a trehalose amorphous matrix, ranging from 0 to 1% (w/w), were prepared, to study the influence of the radical concentration on the DNP properties and to optimize the polarization transfer between the electron and nuclear spins, in order to reach the maximum nuclear polarization. The samples' amorphization was performed simultaneously with the homogeneous mixing of the two components of trehalose and TEMPO, by high energy milling.

Accordingly, differential scanning calorimetry (DSC), Raman (see Figure S1, Supporting Information) and X-ray powder diffraction (XRD) experiments were performed on all the prepared samples, in order to characterize them and to compare the results with the literature.[9] Figure 1 reports the DSC scans recorded at different stages of co-milling of the trehalose-TEMPO system for the specific TEMPO concentration of 0.34%.

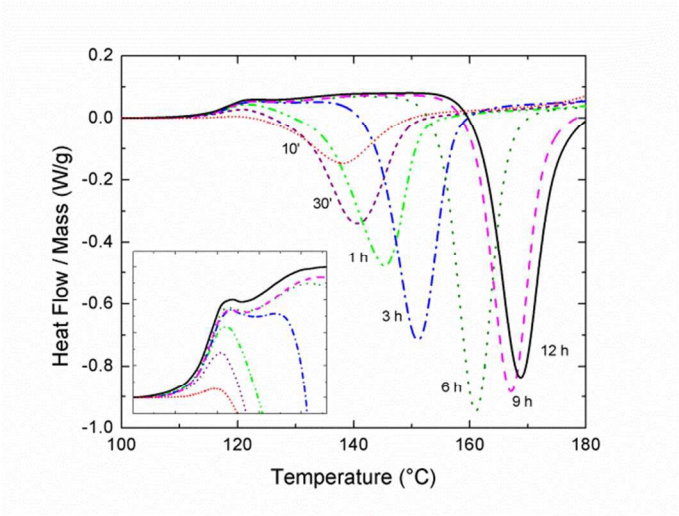

Figure 1. Calorimetric thermograms of trehalose-TEMPO (0.34%) samples prepared by co-milling of the two species for different milling times (10 min - 12 h). The inset shows a zoom of the glass transition region for all the samples.

The thermal profiles show the progressive emergence with milling time of only one glass transition at about Tg = 118 °C (mid-point) as expected from the literature,[10,11] and a subsequent recrystallization. This demonstrate the progressive co-amorphization of the sample and the homogeneity of the final glass solution. Before milling the initial trehalose powder contains microcrystals, commonly of about 100 μm. The amorphous milled sample does not show evidence of micro-, nano-crystals by TEM analysis at 100 nm of magnification (P. Pittia, personal communication). After three hours of milling the disruption of the crystalline phases is complete and the molecules of TEMPO are homogeneously dispersed in the trehalose amorphous matrix. The plasticization effect (progressive decrease of Tg) with increasing TEMPO concentration is shown in Figure S2a, Supporting information. In addition, relevant Bragg peaks of the crystalline sugar phase and Bragg peaks related to TEMPO nanocrystals are found to be absent and co-crystals of the two species are found to be present, in the X-ray diffractogram after 1 h of milling.

The next step is to show that the samples prepared according to the aforementioned procedure can be conveniently used for DNP as they allow a reasonable ^{1}H polarization. Therefore, fully amorphized samples with several radical concentrations were prepared by milling for 12 h and the enhancement of the ^{1}H NMR signal obtained by microwave (MW) irradiation was investigated. Since the ^{1}H signal enhancement (ε, see Supporting Information) varies with the irradiation frequency and the maximum ε_{max} substantially depends on the radical species, the MW frequency yielding the maximum ε in a reference solution of Sodium Acetate (NaAc) with TEMPO was previously determined. The consistency of this preliminary test with the results reported in the literature[12] confirmed the feasibility of the DNP experiment with this setup and made it possible to fix the irradiation frequency to the optimal value for the NaAc reference, at 96.93 GHz in a 3.46 T magnetic field. The polarization build up was measured for all the trehalose samples for $1.7\ K \leq T \leq 4.2\ K$ and the enhancement ε and the polarization time constant (T_{pol}) were estimated from each polarization build-up curve (see Supporting Information). In order to show an example of Dynamic Nuclear Polarization behavior, a comparison is reported between the build-up curves in the sample with 0.5% of TEMPO and in the NaAc-TEMPO reference at T = 4.2 K (Figure 2).

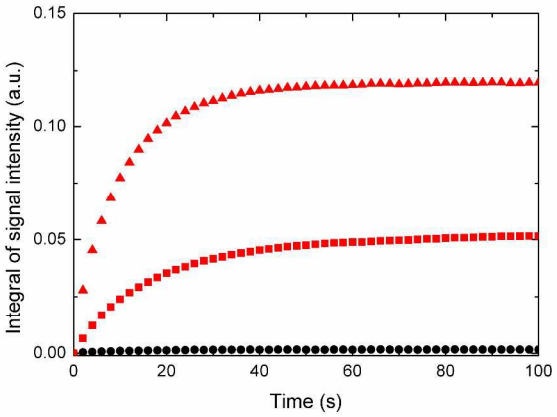

Figure 2. ^{1}H signal build-up curves in NaAc-TEMPO (30 mM, red triangles) and trehalose-TEMPO (0.50%, red squares) samples as a function of time at 4.2 K. The curves obtained during irradiation have been rescaled to the same thermal signal intensity (black dots).

One notices that a significant enhancement of ^{1}H polarization is achieved under MW irradiation, with a build-up time similar to that of the NaAc-TEMPO sample and with a maximum polarization of the same order of magnitude.

To further assess the physical mechanism yielding the observed polarization enhancement, the ^{1}H spectrum for a sample of trehalose and TEMPO (0.64%), obtained by irradiating with MW at 96.93 GHz and sweeping the magnetic field, was measured and found to have the typical shape formerly observed in well-known DNP samples[1,13] (Figure 3). The shape of the curve proves that the enhancement of the ^{1}H NMR investigated is induced by Dynamic Nuclear Polarization, likely in a thermal mixing regime, since the TEMPO electron spin resonance (ESR) linewidth is larger than ^{1}H Larmor frequency.

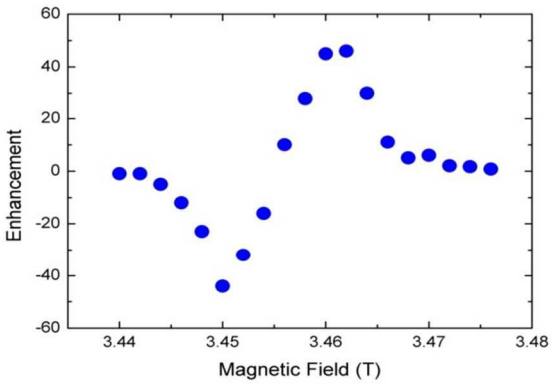

Figure 3. ^{1}H signal enhancement ε in a trehalose-TEMPO sample (0.64%) as a function of the external magnetic field. The temperature and the MW frequency have been set respectively at 1.7-1.75 K and 96.93 GHz.

Figure 4 shows the maximum ^{1}H polarization P achievable via DNP (P = εP0, where P0 is the polarization at thermal equilibrium) as a function of the radical concentration.

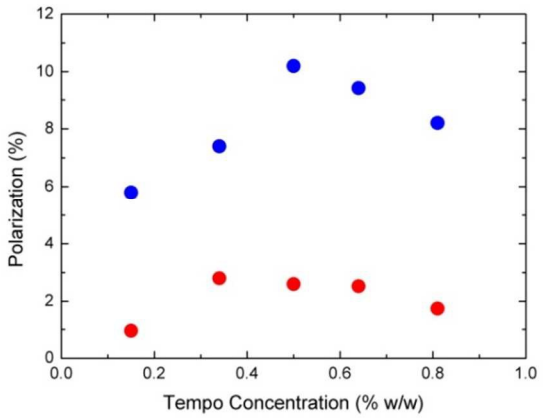

Figure 4. ^{1}H polarization of trehalose-TEMPO as a function of radical concentration at 1.75 K (blue) and 4.2 K (red).

The available data at 1.75 K show an increase in P and a decrease in T_{pol} with radical concentration. Notably, at 1.75 K the polarization P of the 0.5% sample reaches 10% with a polarization time T_{pol} of about 70-100 s. This very first result is quite encouraging since, as has been pointed out above, in the NaAc reference at the same temperature, P ≃ 25%.[12] Moreover, the T_{pol} value measured in the 0.5% sample approaches the one obtained in the NaAc reference (70 s at 1.2 K[12]). Preliminary data at 4.2 K show a similar although smoother trend of P with concentration, eventually leveling off at around 3% (Figure 4) for concentration values between 0.35% and 0.64%.

Conclusions

In conclusion, among the several techniques that may be exploited for obtaining amorphous substrates (e.g., spray drying, freeze drying, co-melting, milling, …)[8], milling turned out to be a valuable route for producing solid hyperpolarized agents, because of its effective procedure, avoiding degradation problems or glass formers with potential toxicological issues. The milling procedure produces an intimate and evenly dispersed mixture of the solid substrate and of the radical species, thus making it possible to perform DNP with high efficiency, as it enables a sizeable interaction between the radical electrons and the nuclei of the substrate. Moreover, mechanochemical manipulation gives forced alloying of target compounds and radicals, even if they are mutually not soluble in the liquid state. The method described is a new "ready to use" formulation of potentially fundamental importance for medical applications and for industrial production.

One further basic advantage of the present preparation method relies on the possibility to analyze and characterize the solid amorphous properties even before the DNP process takes place. This is not possible when conventional methods are used, since the glassy structure is obtained by cooling during the insertion of the sample within the DNP device itself. Indeed, preliminary ESR, Raman and solid state NMR spectroscopic experiments (see Supporting Information) not only confirm the aforementioned results, but also open up the possibility to investigate the effect on the DNP performance of the matrix density of aged glasses or of controlled dispersion of nanocrystals into the amorphous matrix.

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