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Improving Sensitivity of Solid-state NMR Spectroscopy by Rational Design of Polarizing Agents for Dynamic Nuclear Polarization

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§SCS-Metrohm Award for best oral presentation in Physical Chemistry

Abstract: We review our recent efforts to optimize the efficiency of polarizing agents for Dynamic Nuclear Polarization (DNP) in solid-state MAS NMR spectroscopy. We elucidate the links between DNP performance, molecular structure and electronic relaxation properties of dinitroxide biradicals. We show that deuteration and increased bulkiness lead to slower electronic relaxation and in turn to higher DNP enhancements. We also show that the incorporation of solid dielectric particles into the sample is a general method of amplifying DNP enhancements by about a factor of two.

Keywords: DNP · MAS · Nitroxides · TEKPol · TEKPol2

Dominik Kubicki *was born in Poland in 1989. In 2013 he obtained his MSc diploma in chemistry with honours at the Warsaw University of Technology. He then started his PhD in Nuclear Magnetic Resonance under the supervision of Prof. Lyndon Emsley at the Ecole Normale Supérieure (ENS) de Lyon and continued his research at the Ecole Polytechnique Férérale de Lausanne (EPFL). He develops Dynamic Nuclear Polarization methods to increase the sensitivity of NMR by orders of magnitude and allow the studying of surfaces by NMR. He is the author of 11 research articles.*

Chemists use Nuclear Magnetic Resonance spectroscopy (NMR) on a daily basis to characterize the outcome of their synthetic efforts. Solution NMR spectroscopy is a powerful and non-destructive analytical technique in which typically a few milligrams of material are needed to give a good quality proton and carbon spectrum within seconds to minutes. NMR spectroscopy can also be applied to solid materials, which makes it useful for materials science and inorganic chemistry. There are over 120 different NMR-active isotopes that can be more or less routinely probed to reveal structural details of virtually any solid material with atomic resolution, ranging from carefully engineered, inorganic nanostructures to biopolymers, such as bones, wood and feather.[1] However, the technique has a significant downside to it: its sensitivity is relatively low, owing to the intrinsically low polarization of nuclear spins. This becomes a problem for samples in which the molecules of interest are present only at the surface, as is the case in functionalized porous materials or nanoparticles for example, while the bulk of the sample is just an inorganic scaffold for the surface-bound species. In heterogeneous systems, it is at the surface that chemical reactions take place, and hence it is essential to have the capacity to study surfaces with NMR. Such concentration-limited samples, however, cannot be studied using conventional solid-state NMR spectroscopy, since they only contain nano- to micromoles of material, which would require unfeasibly long acquisition times. On top of that, the receptivity of many NMR-active nuclei can be orders of magnitude lower than that of protons and carbon (which itself is 5870 times less receptive than the proton). One of the most efficient ways[2] to tackle the issue of sensitivity is to use Dynamic Nuclear Polarization (DNP).[3]

The idea behind DNP is that one can transfer the comparatively high electron polarization to nuclear spins by creating conditions under which the two systems are coupled and can exchange energy. Such energy matching is provided by microwave irradiation and the maximum theoretical gain in NMR signal intensity can be expressed as γ_e/γ_n, where γ_e and γ_n are the gyromagnetic ratios of the electron and the nucleus, respectively. This expression evaluates, for instance, to 660 for the proton, and 2618 for carbon-13. The unpaired electrons need to be added to the sample before the measurement and to that end one typically uses stable free radicals, for example in the form of stable derivatives of TEMPO dissolved in an organic solvent. The experiment is then carried out using magic angle spinning (MAS) at temperatures of about 100 K, and with *in situ* microwave irradiation. The exact microwave frequency necessary for DNP depends on the physical mechanism that is used to transfer polarization, and at 9.4 T (400 MHz proton Larmor frequency) is on the order of 263 GHz, with several watts of power. This high-frequency and high-power microwave radiation is today generated by a gyrotron and delivered to the NMR probe by means of a corrugated metal

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waveguide. An excellent review of modern DNP instrumentation and the physical mechanisms involved was provided by Maly *et al*.[4] Currently the most efficient DNP mechanism operating under these conditions is the cross-effect (CE), which requires the EPR frequency difference of two dipolar coupled electrons to match the Larmor frequency of the nucleus to which polarization is being transferred. Low temperature provides rigidity to the system, thereby preserving dipolar couplings, which would otherwise be averaged out by molecular motion. The DNP approach has been successfully applied to many areas of research such as functionalized porous materials,[2f,5] structural materials,[6] polymers,[7] nanoparticles,[2f,5d,8] pharmaceuticals,[9] and biomolecular structures[10] that were otherwise previously out of reach.

Here we review recent work in which we have addressed the possibility of making DNP more efficient by 1) improving the existing polarizing agents by rational design, and 2) formulating the sample in a way that leads to better overall microwave propagation. These efforts led to enhancements of about 220 in frozen bulk solutions, and to over 500 in samples mixed with dielectric particles and, most importantly, yielded a deeper understanding of how polarizing agents work.[11]

The notion of making the cross-effect more efficient by tailoring the structure of polarizing agents has been explored by several groups. The key achievements in this respect were the use of nitroxide biradicals (instead of the monoradical TEMPO) in which the interelectron distance is fixed by the length of the linking group. This leads to large electron–electron dipolar couplings and was first shown in 2004 with the introduction of BTnE[12] biradicals and later with TOTAPOL.[13] The cross-effect can be improved still further by making the two nitroxide moieties rigid and orthogonal with respect to one another, since their relative orientation determines the probability of matching the polarization transfer condition.[14] This idea was demonstrated in the bTbK biradical.[15] Another crucial ingredient is the electron spin relaxation time, which determines how efficiently radicals can be saturated with microwaves. At 100 K the main relaxation mechanism relies on lattice vibrations which in turn depend on molecular weight. Our group has shown the importance of this parameter by introducing the bulky (and so heavy), rigid biradicals bCTbK and TEKPol which prove to have long electron spin relaxation times, which gave unprecedented DNP efficiency with enhancements of over 200, and with high performance even at temperatures considerably higher than 100 K.[16]

Up to this point, most of the highest performance radicals developed were not soluble in water. One way to solubilise them in aqueous systems was to use micelles.[17] The need for water-soluble polarizing agent was addressed in 2013 by Sauvée *et al*. who introduced the bulky PyPol and AMUPol radicals, with the latter typically yielding enhancements of 250 in water-based solvents.[16b,18]

We recently demonstrated the relationship between fine structural modifications of polarizing agents featuring the characteristics described above.[11a] To this end, we assembled a large collection of biradicals based on several backbones and compared their performance under similar conditions (9.4 T magnetic field, similar volume of bulk solutions in 1,1,2,2-tetrachloroethane (TCE) flash frozen at 100 K to form a good glass, all experimental details can be found in the original paper[11a]). The study suggests that the apparent performance of the best radicals may now be limited by other factors, and one such factor is microwave propagation through the sample. In fact, in a different study we showed that this limit can be overcome by simply mixing the bulk solution with solid dielectric particles which act as microwave radiation scatterers. Moreover, we showed that certain sample regions experience the theoretical maximum enhancement of 660 since the microwave field distribution in the sample is not homogeneous.[11b]

Results and Discussion

Fig. 1 shows the structures of the investigated polarizing agents categorized according to their backbone. There are five backbones, named after the simplest radical featuring them: bTurea, bCTurea (only as derivatives), PyPol and bTbK. The additional TEKPol series is based on the bTbK backbone with additional spirocyclohexyl groups on the radical moieties. In the urea-based radicals we chose two strategies to modify the molecules: 1) by adding N-substituents in the urea linker such as CH_3 and CD_3 groups, PEG chains of different length and by linking the two nitrogen atoms with rigid linkers, and 2) by adding bulky substituents at the α position of the nitroxide moiety. In the bTbK series only the latter strategy was used.

Fig. 2 shows the DNP enhancements measured on the solvent (TCE) peak in frozen 16 mM bulk solutions. A clear trend emerges: all derivatives of bTurea perform better than bTurea itself. Derivatives of bCTurea perform on average better than derivatives of bTurea. Derivatives of PyPol have on average similar performance to derivatives of bCTurea. The TEKPol series consistently outperforms the other series.

Correlation between Electronic Relaxation and DNP Performance

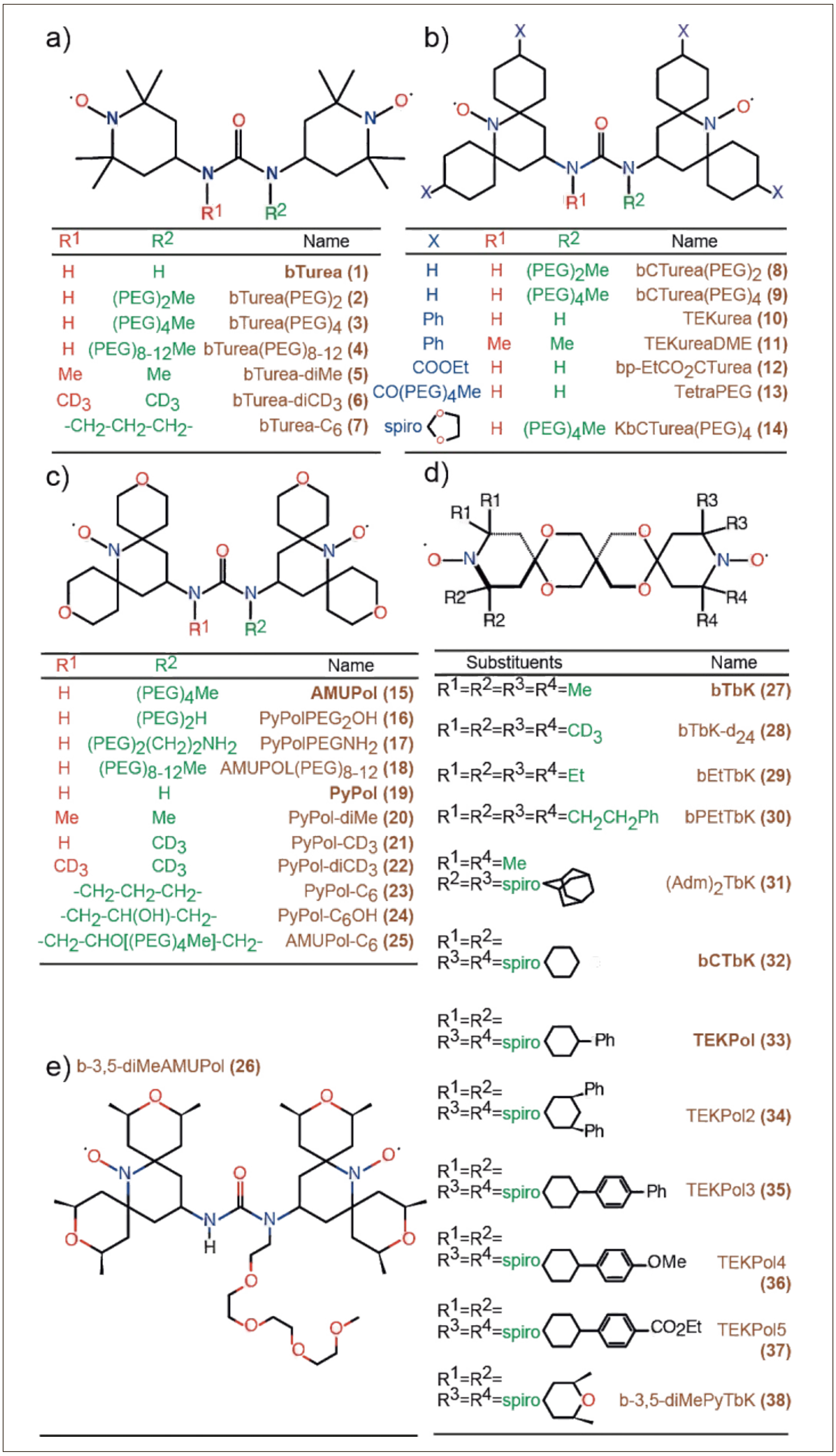
Fig. 3 shows the DNP enhancements of chosen polarizing agents from the bTurea, PyPol and bTbK series as a function of their electronic relaxation parameters. We use here the electron saturation factor $T_{1e} \cdot T_{2e}$, which is a convenient phenomenological parameter related to the efficiency of continuous wave (CW) saturation.[19] We found that there is a clear correlation between DNP enhancements and the saturation factor in the bTbK series: the higher the saturation factor the higher the DNP enhancement. This trend is also present but less pronounced in the PyPol series. In the bTurea series the correlation is difficult to assess with only four data points.

Effect of Deuteration

It is well known in the EPR literature that electronic relaxation depends on proton concentration in the frozen solvent below 70 K and can be slowed down by deuterating the solvent.[20] In fact, improved DNP enhancements were observed in partly deuterated solvents.[21] Perras *et al.* have recently shown that deuteration of bTbK derivatives slows down their electron relaxation and leads to up to 70% higher DNP enhancements. It also reduces the number of protons in the immediate vicinity of the polarization source (the so-called 'spin diffusion barrier'), which do not contribute to the observable signal and can be thought of as spoiling the useful polarization that reaches out to the solvent.[22] Methyl groups are particularly detrimental in this respect since they act as relaxation sinks and we expected them to strongly affect electronic relaxation at 100 K.[20b] We confirmed this by comparing protonated and deuterated bTurea-diMe (**5**, **6**) and PyPol-diMe (**20**, **22**). The deuterated varieties gave slightly higher DNP enhancements (by 27% and 15%, respectively). The T_{1e} and T_{2e} of **22** were 50% and 26% longer, respectively, than in **20**. But there is a downside to deuteration, too: deuterated radicals have longer DNP build-up times, which imposes slower repetition rates when signal averaging. This trade-off is well illustrated in the deuterated version of bTbK (bTbK-d_{24}, **28**) in which all the methyl groups are deuterated. It yielded 20% higher DNP enhancement and 50% higher saturation factor than bTbK (**27**), but the build-up time increased by 80%.

Effect of Crowding around the Unpaired Electron

Sterical hindrance around the unpaired electron in polarizing agents is a factor that had previously never been taken into account. A recent report on a nitroxide with ethyl groups in the α position, which resists reduction by ascorbate inspired us to look at this in more detail.[23] To that end we made a bTbK derivative (**29**) with ethyl groups in the α position. The saturation factor of this derivative (**29**) was found to be about three times higher than the one of bTbK,[16a] but unexpectedly the DNP enhancement is two times lower. This result was confirmed on two different preparations of the radical, and we are currently looking at analogous systems to elucidate this effect. On the other hand, a radical with phenylethylene moieties in the α position (bPEtTbK, **30**) performs similarly to TEKPol (**33**) and TEKPol2 (**34**). In an attempt to further increase the bulkiness and rigidity we prepared a bTbK analogue with adamantyl groups in the α position (**31**). In this case the DNP enhancement was 55% higher than for bTbK.



R^1	R^2	Name
H	H	**bTurea (1)**
H	$(PEG)_2Me$	bTurea$(PEG)_2$ **(2)**
H	$(PEG)_4Me$	bTurea$(PEG)_4$ **(3)**
H	$(PEG)_{8\text{-}12}Me$	bTurea$(PEG)_{8\text{-}12}$ **(4)**
Me	Me	bTurea-diMe **(5)**
CD_3	CD_3	bTurea-diCD_3 **(6)**
$-CH_2-CH_2-CH_2-$		bTurea-C_6 **(7)**

X	R^1	R^2	Name
H	H	$(PEG)_2Me$	bCTurea$(PEG)_2$ **(8)**
H	H	$(PEG)_4Me$	bCTurea$(PEG)_4$ **(9)**
Ph	H	H	TEKurea **(10)**
Ph	Me	Me	TEKureaDME **(11)**
COOEt	H	H	bp-EtCO_2CTurea **(12)**
$CO(PEG)_4Me$	H	H	TetraPEG **(13)**
spiro	H	$(PEG)_4Me$	KbCTurea$(PEG)_4$ **(14)**

R^1	R^2	Name
H	$(PEG)_4Me$	**AMUPol (15)**
H	$(PEG)_2H$	PyPolPEG$_2$OH **(16)**
H	$(PEG)_2(CH_2)_2NH_2$	PyPolPEGNH$_2$ **(17)**
H	$(PEG)_{8\text{-}12}Me$	AMUPOL$(PEG)_{8\text{-}12}$ **(18)**
H	H	**PyPol (19)**
Me	Me	PyPol-diMe **(20)**
H	CD_3	PyPol-CD_3 **(21)**
CD_3	CD_3	PyPol-diCD_3 **(22)**
$-CH_2-CH_2-CH_2-$		PyPol-C_6 **(23)**
$-CH_2-CH(OH)-CH_2-$		PyPol-C_6OH **(24)**
$-CH_2-CHO[(PEG)_4Me]-CH_2-$		AMUPol-C_6 **(25)**

Substituents	Name
$R^1=R^2=R^3=R^4=$Me	**bTbK (27)**
$R^1=R^2=R^3=R^4=CD_3$	bTbK-d_{24} **(28)**
$R^1=R^2=R^3=R^4=$Et	bEtTbK **(29)**
$R^1=R^2=R^3=R^4=CH_2CH_2Ph$	bPEtTbK **(30)**
$R^1=R^4=$Me $R^2=R^3=$spiro	$(Adm)_2$TbK **(31)**
$R^1=R^2=$ $R^3=R^4=$spiro	**bCTbK (32)**
$R^1=R^2=$ $R^3=R^4=$spiro –Ph	**TEKPol (33)**
$R^1=R^2=$ $R^3=R^4=$spiro Ph Ph	TEKPol2 **(34)**
$R^1=R^2=$ $R^3=R^4=$spiro –Ph	TEKPol3 **(35)**
$R^1=R^2=$ $R^3=R^4=$spiro –OMe	TEKPol4 **(36)**
$R^1=R^2=$ $R^3=R^4=$spiro –CO_2Et	TEKPol5 **(37)**
$R^1=R^2=$ $R^3=R^4=$spiro O	b-3,5-diMePyTbK **(38)**

Fig. 1. Structures and names of the radicals described in this review: (a) the bTurea series, (b) the bCTurea series, (c, e) the PyPol series, (d) the bTbK series. The prefix *spiro* indicates a junction through just one carbon atom. *PEG* indicates a $-CH_2CH_2O-$ unit. Adapted from ref. [10], with permission.

Effect of PEG Chain Length in Urea-based Radicals

PEG chains are known to improve solubility of polarizing agents, the most prominent example of which is the state-of-the-art water-soluble biradical AMUPol.[10] To get an estimate of the optimal PEG chain length we prepared several bTurea and PyPol derivatives with different numbers of PEG units. Comparatively higher enhancement were observed for 2–4 PEG units (**2**, **3**, **8**, **9**, **15**) than for a longer chain (8–10 units, **4**). We also found that long PEG chains actually shorten electronic relaxation. We observed a similar effect in TetraPEG (**13**).

Effect of Increased Bulkiness in bTbK-based Radicals

As pointed out previously, it is now well established that more bulky radicals give higher saturation factors and better DNP performance. To probe this dependence in more detail we introduced additional decorating groups to TEKPol (**33**). The regioisomers TEKPol2 (**34**) and TEKPol3 (**35**) possess an extra phenyl ring compared to TEKPol. In **35** the introduction of the phenyl ring in the *para* position does not improve the saturation factor (instead we saw a reduction of about 10%) while in **34** the introduction of two phenyl rings into the 3,5 position increases the saturation factor by 50%. As expected, this leads to higher DNP enhancement and TEKPol2 (**34**) yielded the highest DNP enhancement (222±22) obtained so far under such conditions. The introduction of a OMe (**36**) or a COOEt (**37**) group into the phenyl ring of TEKPol reduces DNP enhancements by 50% or 23%, respectively, and the saturation factor of **36** is reduced by 50%. We cannot explain it by invoking the fast relaxation of methyl groups, since radical **38** has them too and still yields a high enhancement. This result shows that fine tuning of DNP performance in dinitroxide biradicals is a complex problem.

Effect of Mixing Solid Particles with the Sample

Fig. 4 compares DNP enhancements obtained in bulk solutions (as described above) and in solutions mixed with solid particles (with solid volume fraction of about 0.6). In the latter case the enhance-

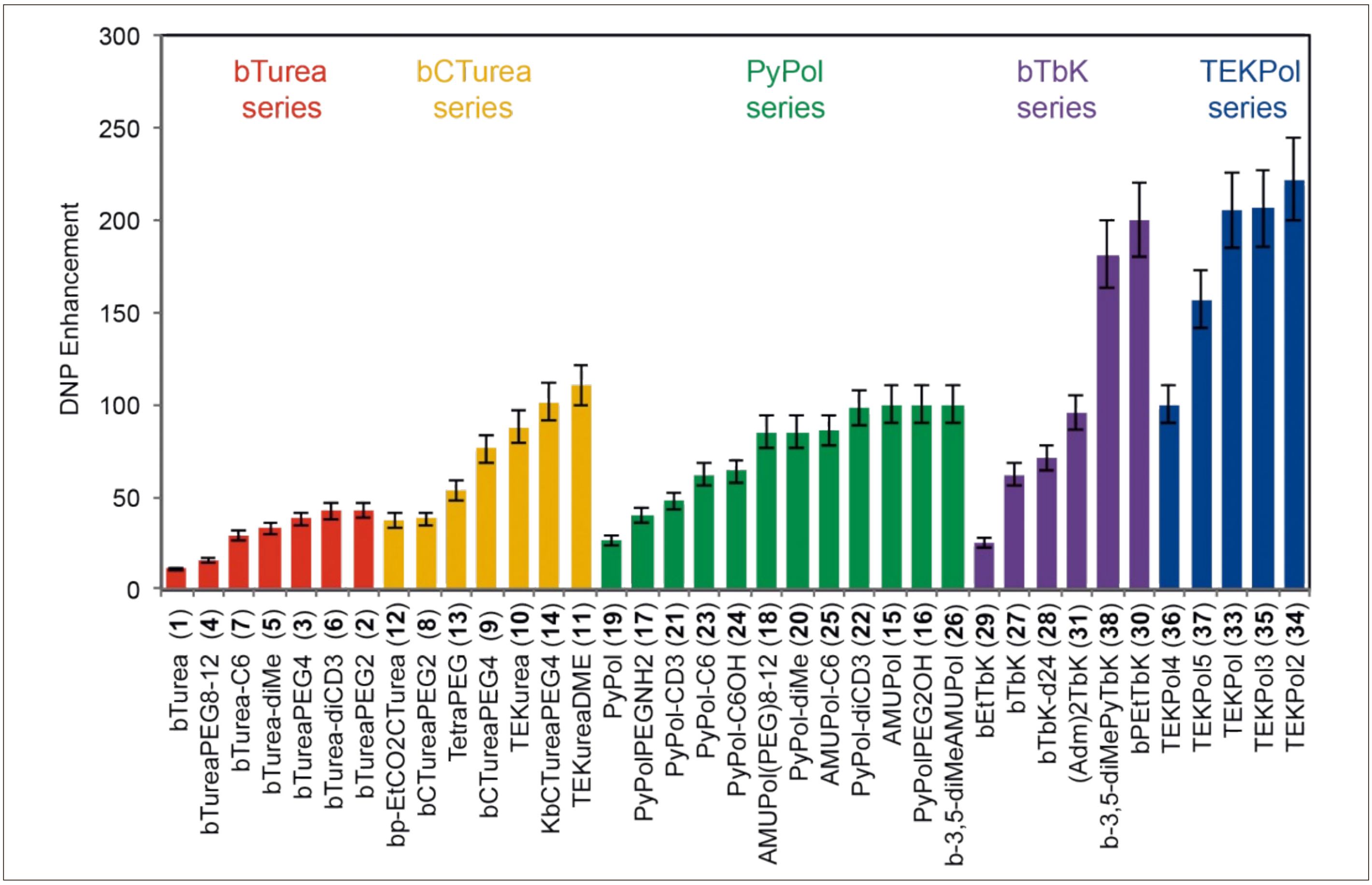


Fig. 2. DNP enhancement of 16 mM bulk solutions of radical in TCE. Adapted from ref. [10], with permission.

ment is always higher and can reach as much as 250% of the bulk reference value. We observed this effect both in the organic solvent TCE and in water-based mixtures, as well as with many different (inorganic and organic) particles. We probed the effect of their average size and found it to be of comparatively little importance. In attempt to explain its origin we carried out finite-element simulations of microwave propagation in model samples. The results suggested the phenomenon arises because dielectric materials create microstructures that scatter the incident beam in a way that leads to a significant redistribution of the electromagnetic field inside the sample. This then leads to an increased average microwave field experienced by the radicals.[11b]

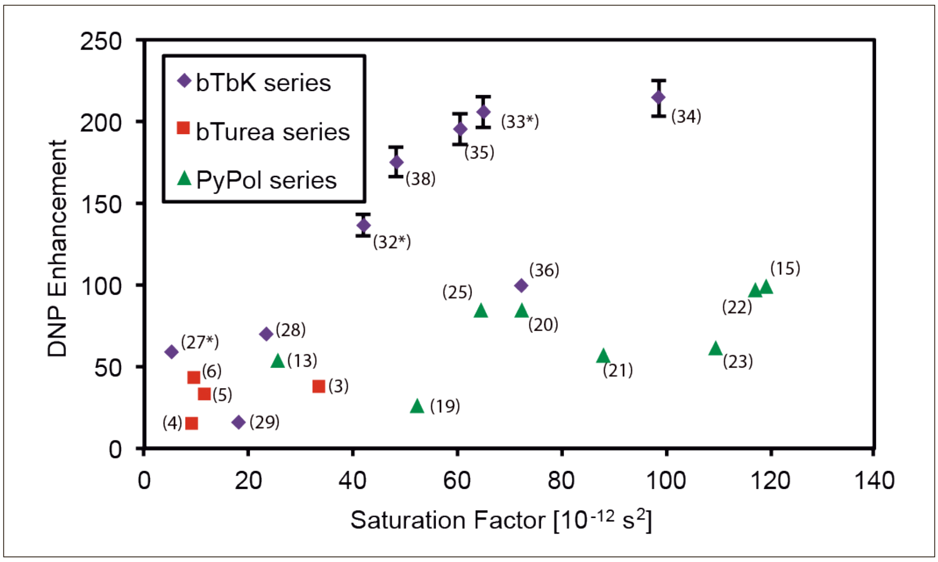


Fig. 3. DNP enhancement as a function of saturation factor ($T_{1e} \cdot T_{2e}$) for selected compounds from the bTurea, PyPol and bTbK series (TCE, 16 mM). The data points marked with an asterisk are taken from the previous study by Zagdoun *et al.*[16b] Error bars are shown where larger than the symbols. Adapted from ref. [10], with permission.

Conclusions

In conclusion, our recent work has confirmed that DNP enhancements are strongly correlated to electronic relaxation properties of polarizing agents, with slower relaxation leading to higher enhancements. The work has shown that deuteration and increased bulkiness are advantageous in this respect, although deuteration leads to longer build-up times which defeats the sensitivity improvement. We discovered that further increase of bulkiness in TEKPol derivatives leads to little or no improvement which suggests that other physical phenomena may be limiting the observed enhancements at 9.4 T and 100 K. This is confirmed by our other recent study in which we show that enhancements close to the theoretical maximum are achievable by simply mixing the sample with solid particles such as KBr or sapphire. That said, we highlight that one of the new radicals, dubbed TEKPol2, performs slightly better than the previous state-of-the-art radical TEKPol when used in bulk solutions. We

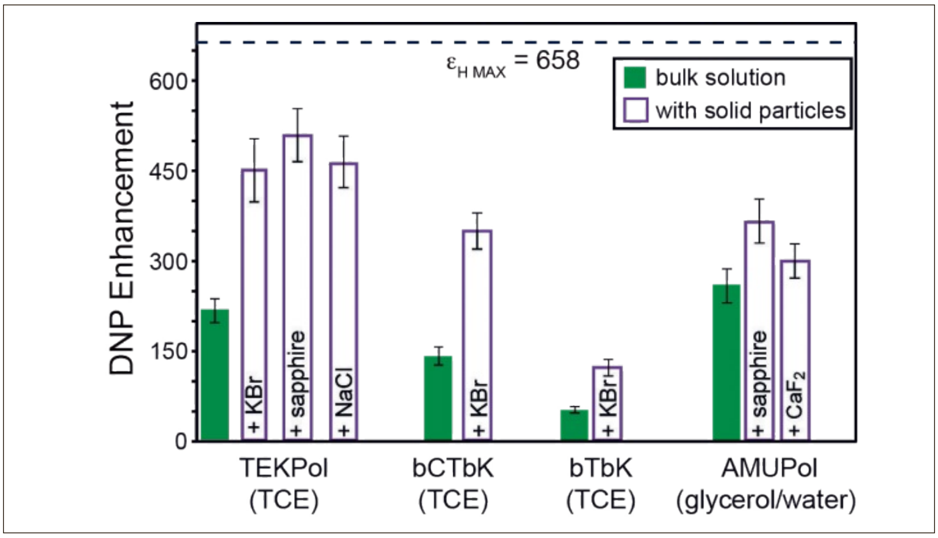


Fig. 4. DNP enhancements observed in bulk solutions (green columns, TCE, 16 mM) and when the solution is mixed with solid particles (open columns, solid type indicated inside). The dashed line indicates the theoretical maximum achievable proton enhancement of 658. Adapted from ref. [10], with permission.

are currently addressing the issue of relatively lower enhancements obtained with these radicals at higher magnetic fields.

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