Kinetics of Covalent Bond Formation of Sulfonamide with Soil Humic Acid: ESR Investigations with Nitroxide Spin Label

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Sulfonamides in soil

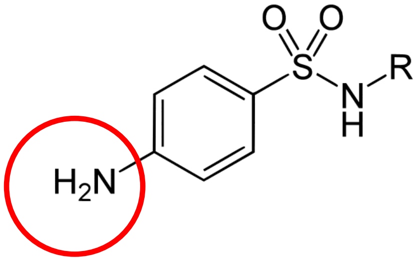
- Sulfonamides are veterinary antibiotics, which are widely used in animal husbandry for treatment of infections.
- After application of manure of treated livestock to soil, sulfonamides interact with organic soil components, e.g. by reversible sorption or irreversible formation of non-extractable residues.
- The latter is attributed to physical entrapment or/and covalent binding to soil organic matter.

Outline

- Rapid formation of non-extractable residues of sulfonamides with soil
- Nitroxide spin probing using Electron Spin Resonance (ESR) spectroscopy
- Interaction of spin-labeled sulfapyrimidine analogue (SA) with Leonardite humic acid (LHA)
- Covalent binding or physical entrapment?
- Kinetics of covalent binding of SA to LHA in the presence of extracellular fungal laccase
- Summary and outlook

Non-extractable residues (NER)

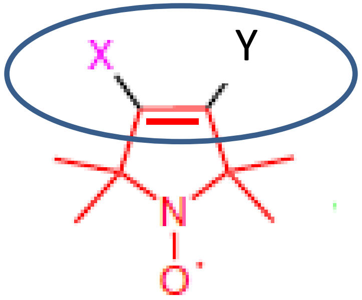
- Rapid loss of extractability of sulfonamides was observed in soil (Barriuso et al. 2008, Müller et al. 2013).
- Sulfonamides are covalenty bound to soil humic acids via the aniline nitrogen (Bialk et al. 2005, 2007, 2008; Gulkowska et al., 2012, 2013).



- Nucleophilic addition reaction to quinones is responsible for covalent bond formation of aromatic amines (Weber et al. 2001; Thorn and Kennedy, 2002, Matthies et al., 2016).
- Aromatic amines are building blocks for pesticides, biocides, pharmaceuticals and other classes of synthetic chemicals.

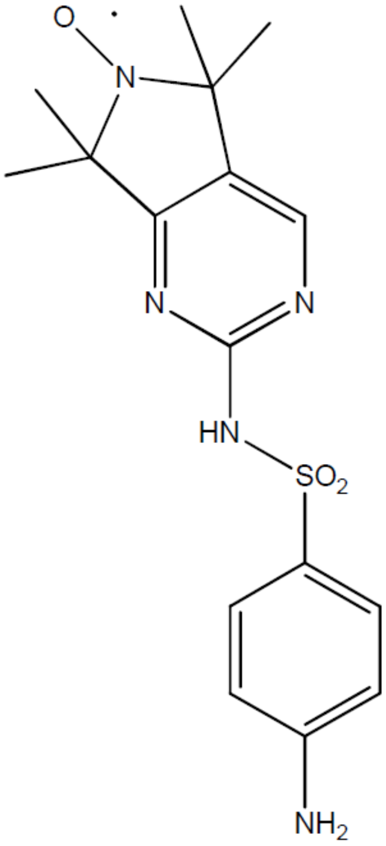
Nitroxide spin probing

- Stable nitroxide radicals are successfully used in molecular biology since many years for, e.g. protein-membran interaction, protein structure analysis.
- Xenobiotics or their functional groups can be labelled with nitroxide radicals to study their interaction in complex molecular environments with Electron Spin Resonance (ESR) spectroscopy.

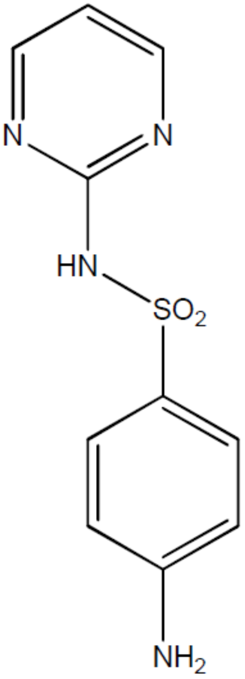


- Spin probe signals depend on the molecular environment (polar and non-polar) and the binding state (covalent, H-bond).
- Reversible interaction can be distinguished from irreversible reaction (covalent bond).

Spin-labeled sulfapyrimidine analogue (SA)



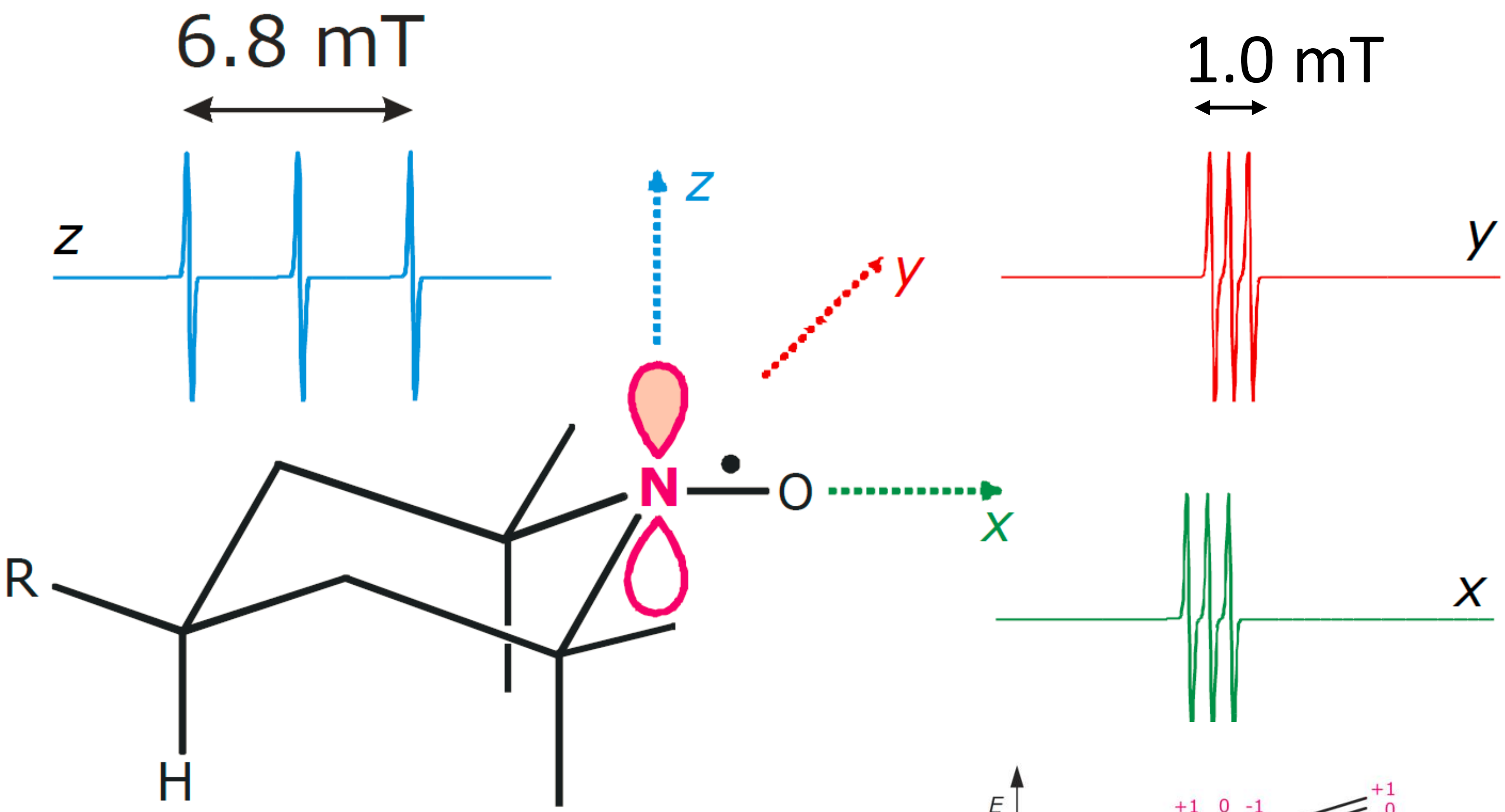
HO-4888

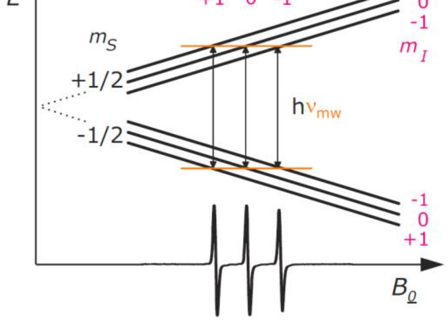


Sulfapyrimidine

G. Úr et al. (2017) Synthesis, DOI: 10.1055/s-0036-1589034

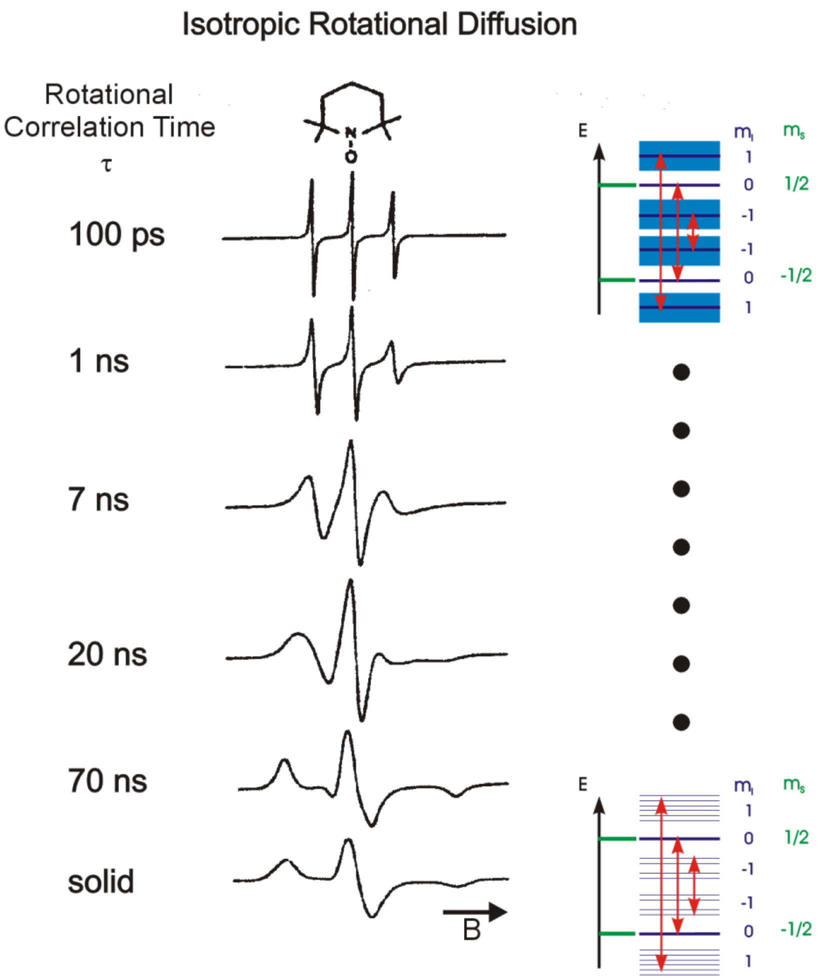
Nitroxide ESR spectrum





Hyperfine splitting of the energy levels
of an unpaired electron with a ^{14}N nucleus
in a magnetic field

Motional Narrowing



fast motional limit:

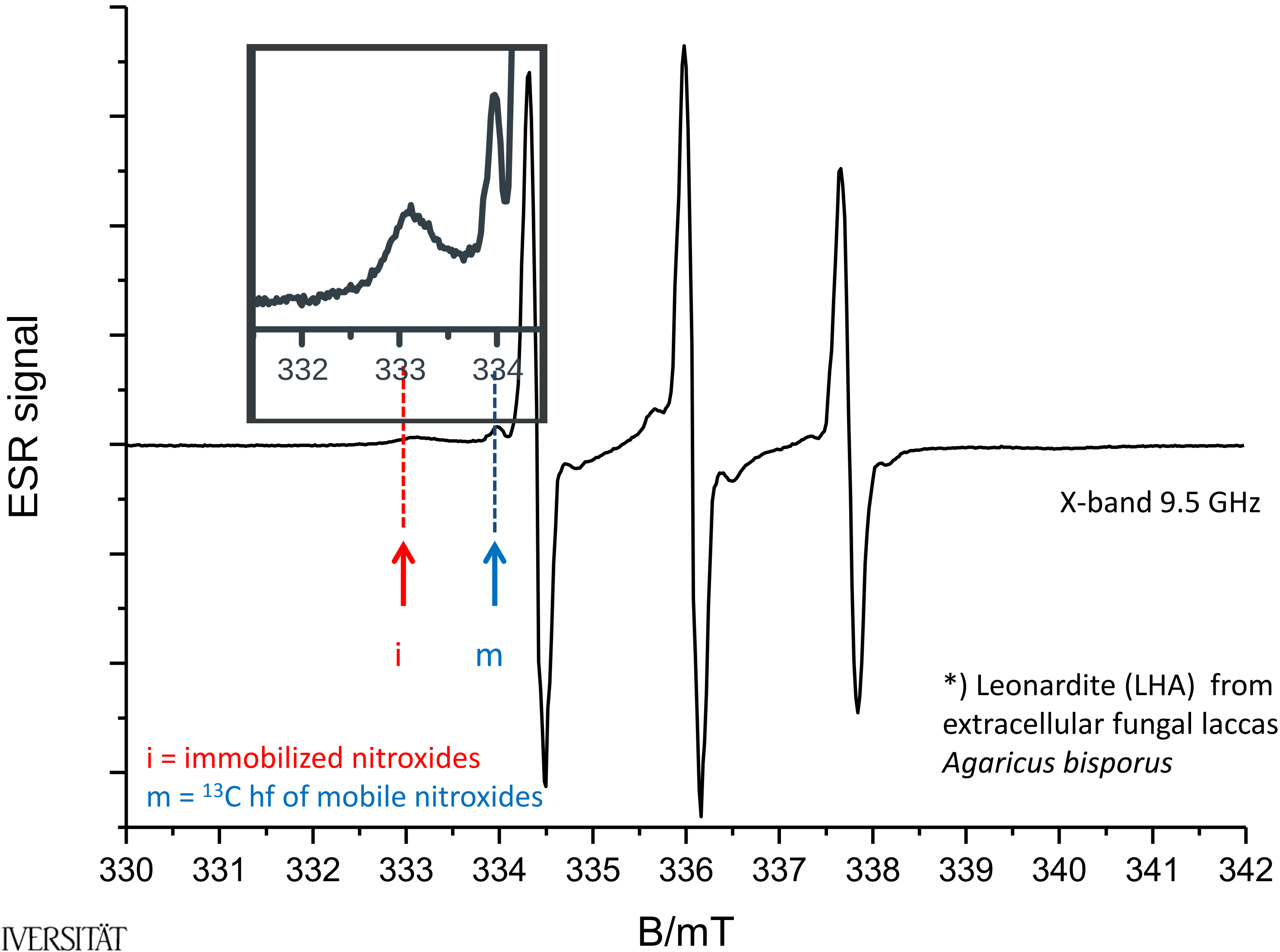
anisotropy is averaged

slow motion

rigid limit:

anisotropy of hyperfine interaction

SA spin probing with humic acid*



X-band 9.5 GHz

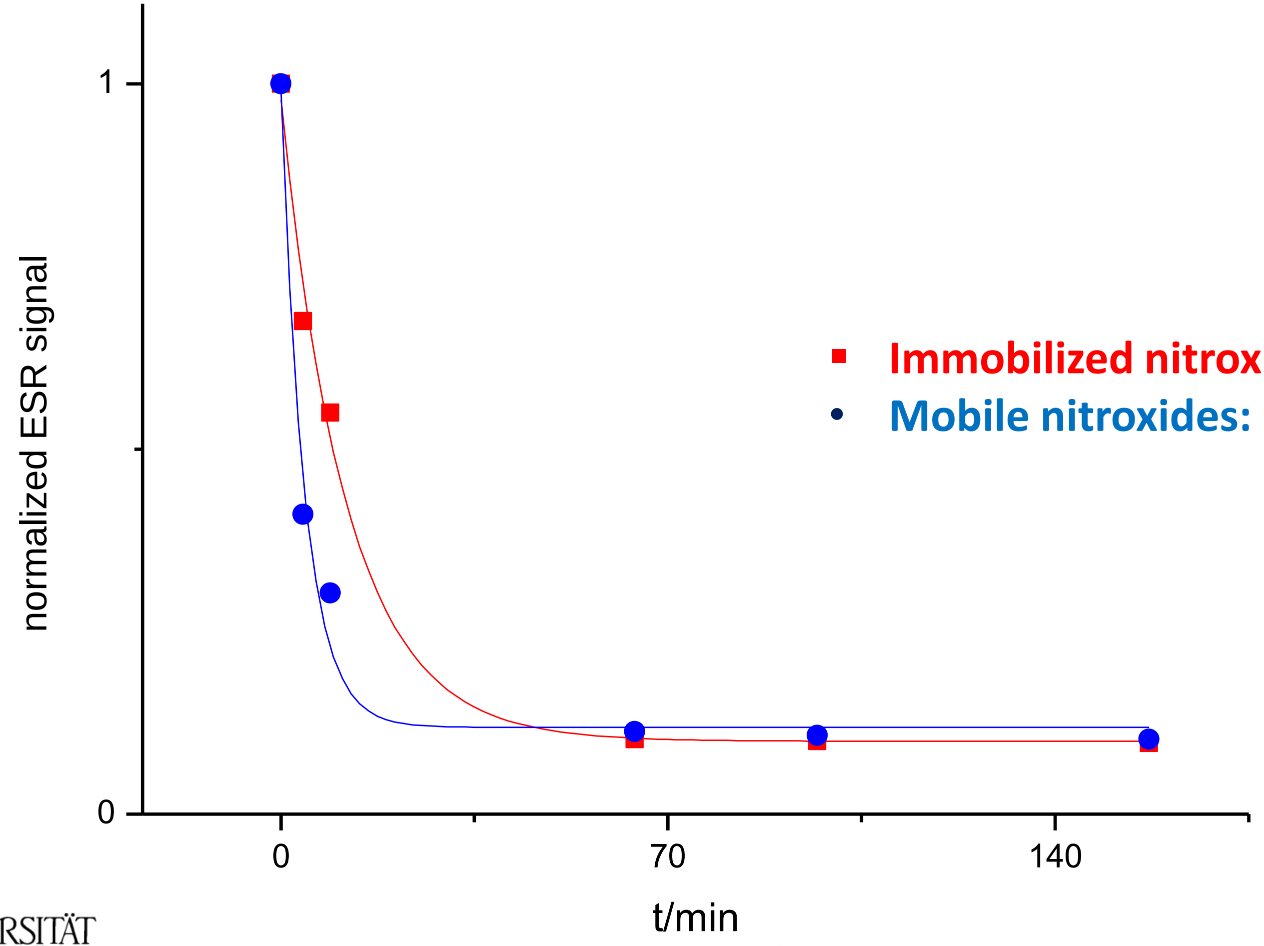
i = immobilized nitroxides

m = ^{13}C hf of mobile nitroxides

*) Leonardite (LHA) from IHSS with extracellular fungal laccase from *Agaricus bisporus*

Covalent binding or physical entrapment?

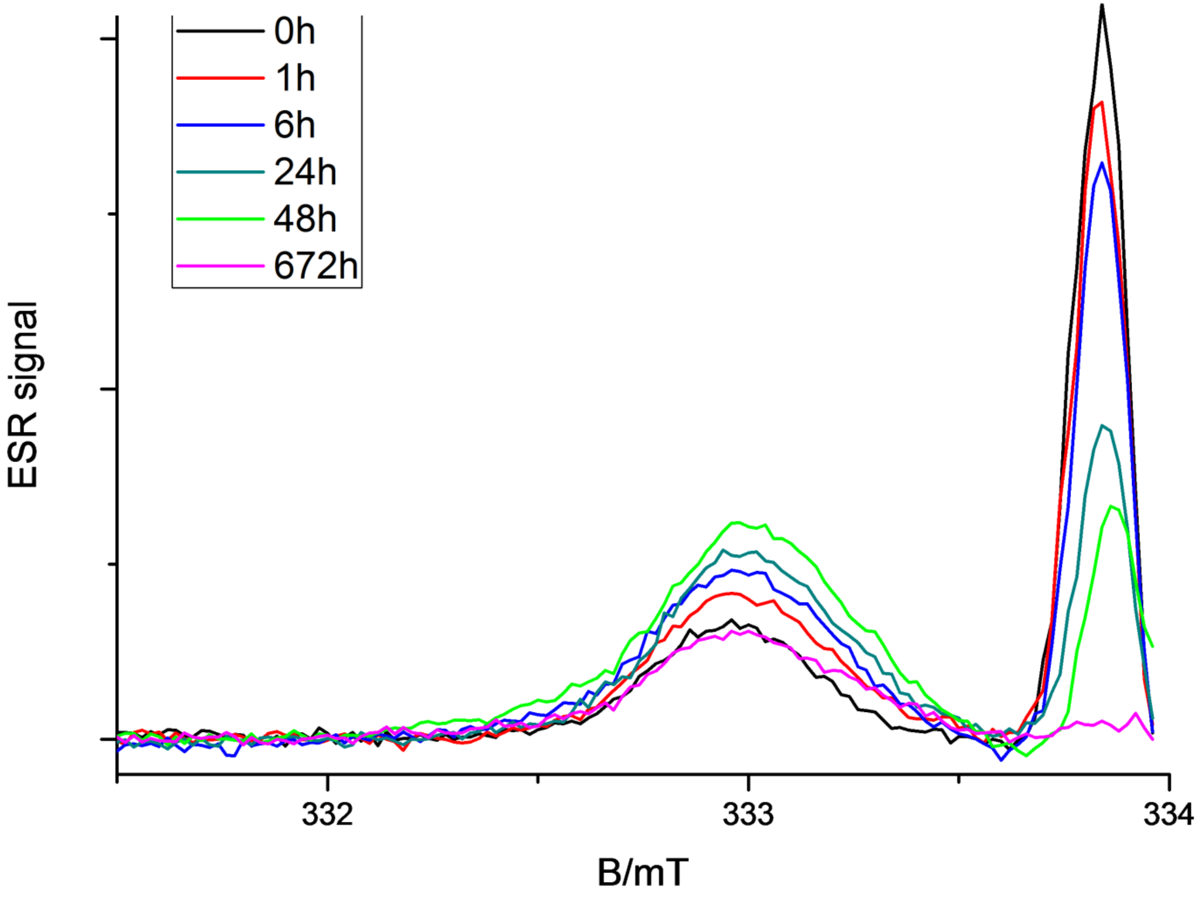
Reduction of nitroxide spin with antioxidant sodium ascorbat



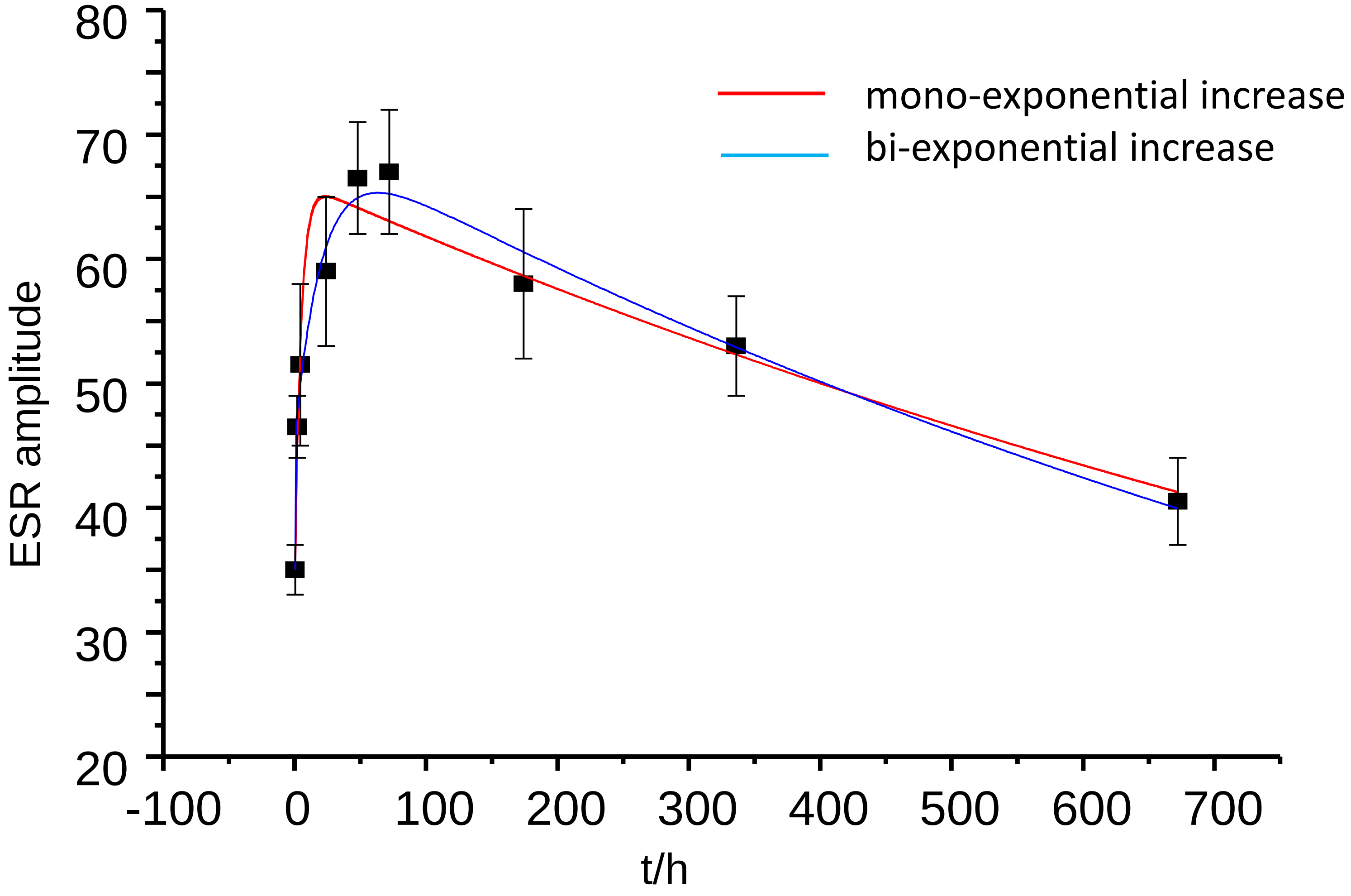
■ **Immobilized nitroxides: $t_{1/2}$ = 12 min**

● **Mobile nitroxides: $t_{1/2}$ = 4.5 min**

Covalent binding of SA to LHA



Kinetics of covalent binding to LHA



Kinetic modelling

One or two parallel binding reactions overlayed with spin reduction

Model	$t1_{1/2}$ [h]	$t2_{1/2}$ [h]	$t3_{1/2}$ [h]	X^2	R^2
Mono-exponential	4.80 ± 1.70	—	1419 ± 273	12.5	0.89
Bi-exponential	0.21 ± 0.04	21.02 ± 8.16	1195 ± 168	5.39	0.956
Bi-exponential Anilino-NO*)	0.043 ± 0.040	1.15 ± 0.11	n. d.	4.53	0.998

$t1_{1/2}$, $t2_{1/2}$ = half-times for covalent binding reactions
$t3_{1/2}$ = half-time of overall spin reduction
*) Matthies et al. (2016) Appl Magn Reson 47:627–641

Summary and Outlook

- The ESR spectrum of a newly synthesized nitroxide spin-labeled sulfapyrimidine analogue (SA) shows strong interaction with soil humic acid.
- Treatment with the antioxidant sodium ascorbat reveals covalent binding of SA to LHA and not physical entrapment.
- An initial rapid binding reaction with a half-time of 12.6 minutes and a slower one with a half-time of 21 hours are observed presumably with reactive quinone moeties of LHA in the presence of extracellular fungal laccase.
- A much slower reaction with a half-time of 50 days is attributed to paramagnetic loss reaction (reduction of nitroxide spin).
- Next step is the investigation of SA interaction with soil clay suspensions and mixtures with humic acids.

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Many thanks for your attention!

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