

Electronic Supplementary Information (ESI)

Solubilisation of a 2,2-diphenyl-1-picrylhydrazyl radical in water by β -cyclodextrin to evaluate the radical-scavenging activity of antioxidants in aqueous media

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Experimental details

Materials

Commercially available reagents, such as 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH[•]), β -cyclodextrin (β -CD), ascorbic acid (AscH₂), Trolox, buffer salts and organic solvents, were the best available purity and used without further purification unless otherwise noted. Water used in this study was freshly prepared with a Milli-Q system (Millipore Direct-Q UV 3). The solubilisation of DPPH[•] to water using β -CD was carried out by the procedure described in the text.

Spectral and kinetic measurements

UV-vis spectra were recorded on an Agilent 8453 photodiode array spectrophotometer. The rates of the scavenging reaction of the β -CD-solubilised DPPH[•] (DPPH[•]/ β -CD) in a phosphate buffer (0.1 M, pH 7.4) by water soluble antioxidants (AscH₂ and Trolox) were determined by monitoring the absorbance change at 527 nm due to DPPH[•]/ β -CD ($\epsilon = 1.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) using a stopped-flow technique on a UNISOKU RSP-1000-02NM spectrophotometer. The pseudo-first-order rate constants (k_{obs}) were determined by a least-squares curve fit using an Apple MacBook Pro personal computer. The first-order plots of $\ln(A - A_{\infty})$ vs. time (A and A_{∞} are denoted as the absorbance at the reaction time and the final absorbance, respectively) were linear until three or more half-lives with the correlation coefficient $\rho > 0.999$.

Theoretical calculations

Density functional theory (DFT) calculations of β -CD, DPPH[•], DOPPH[•], and their inclusion complexes (DPPH[•]/ β -CD and DOPPH[•]/ β -CD) were performed with Gaussian 09 (Revision A.02, Gaussian, Inc.).^{S1} The calculations were performed on a 32-processor QuantumCubeTM at the UB3LYP/3-21G level of theory for DPPH[•], DOPPH[•] and their complexes and the RB3LYP/3-21G for β -CD.^{S2} The polarizable continuum model (C-PCM), parameterized for water as a solvent, was applied during the geometry optimisation step. Graphical outputs of the computational results were generated with the *GaussView* software program (ver. 3.09) developed by Semichem, Inc.^{S3}

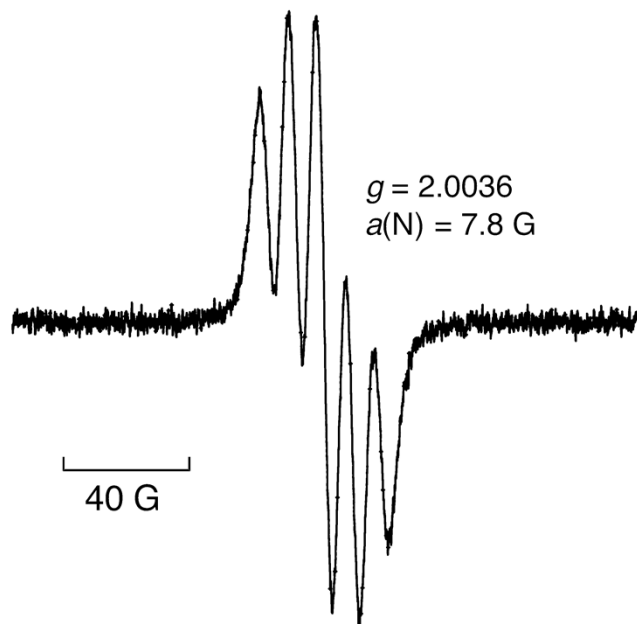
EPR measurements

EPR spectra of DPPH[•] and the inclusion complex (DPPH[•]/ β -CD) were recorded on a JEOL X-band spectrometer (JES-RE1XE) with an attached variable temperature apparatus. The EPR spectra were measured at 77 (Fig. S1) and 298 K (Fig. 4). The magnitude of modulation was chosen to optimise the resolution and the signal-to-noise (S/N) ratio of the observed spectra under non-saturating microwave conditions. The g values and the hyperfine coupling constants were calibrated with a Mn²⁺ marker.

References

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(a) DPPH[•]/β-CD in H₂O



(b) DPPH[•] in MeOH

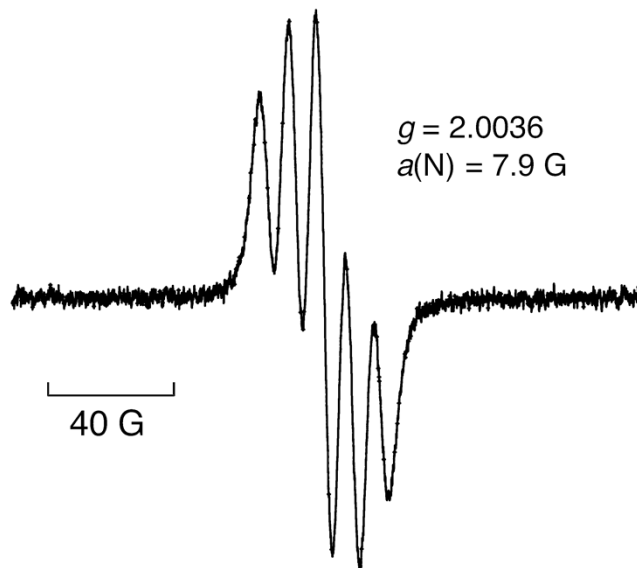


Fig. S1 EPR spectra of (a) DPPH[•]/β-CD ($1.0 \times 10^{-5} \text{ M}$) in distilled water at room temperature and (b) DPPH[•] ($1.0 \times 10^{-5} \text{ M}$) in MeOH at room temperature.