Antioxidant Hydrogen Atom Transfer by Humic Acids & HA-NanoHybrids:

Understanding the fundamentals & connecting it to technology/industry

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21/6/2017

OUTLINE

NATURAL ORGANIC MATTER RESEARCH

1. Antioxidant (background)

Electron transfer

Hydrogen Atom Transfer (HAT)

2. Polyphenols-Humics

H^+-shuttling (known)

e^- donation capacity (known)

Radical stabilization capacity (known)

Hydrogen Atom Transfer (HAT) capacity (unknown)

3. HA-nanohybrids

Motivation

HA-Nanotechnology-Industrial interest

New dimension in fundamental research:: HA-Antioxidant

4. Results

HA/HA@SiO2 Antioxidant Thermodynamics

5. What we learn-Conclusions-Challenges

Antioxidants in cosmetics

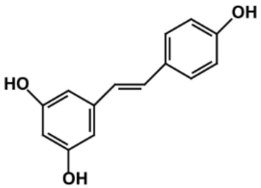
- Skin care
  - UV irradiation forms radicals[1]
  - Radicals induce skin aging, wrinkles
  - Facial photo-aging
    - Antioxidants prevent aging by inhibiting radica activity
    - Antioxidant compounds (e.g. Q10, caffeine)

[1] Griffiths. *Drugs & Aging* **14**, 289 (1999).

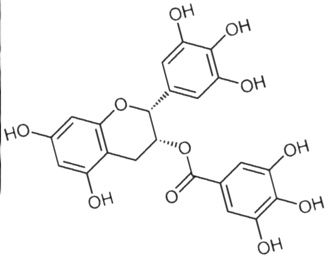
Antioxidants in food & nutrition

- Exist naturally in foods[1]
  - Fruits, vegetables, wine, tea, coffee,..
- Recent years in supplements too
  - e.g. Resveratrol, green tea extract…
- Promote immune system health[2]
  - Prevent diseases

Resveratrol



Epigallocatechin gallate

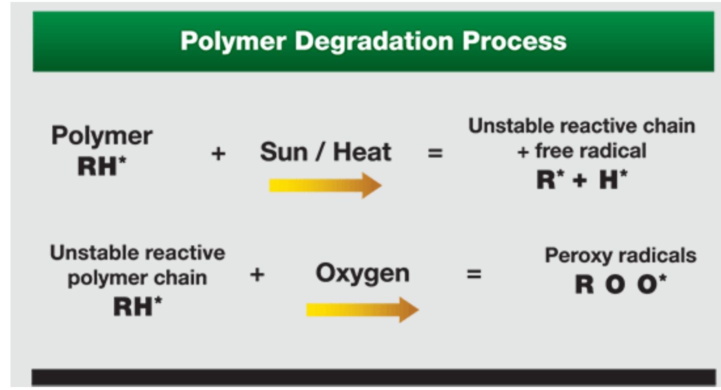


www.dsm.com

[1] Halliwell. *Annu. Rev. Nutr.* **16**, 33(1996).
[2] Press release DSM Nutritional Products. Kaiseraugst, 3 October 2012.

Antioxidants in polymer-industry

- UV irradiation, thermal stress can induce radicals in the polymers[1]
- Radicals degrade the polymer
- Loses performance
  - Mechanical strength
  - Coloristic changes
- Antioxidant additives



[1] Haider, Karlsson. *Polym. Degrad. Stab.* **64**, 321 (1999).

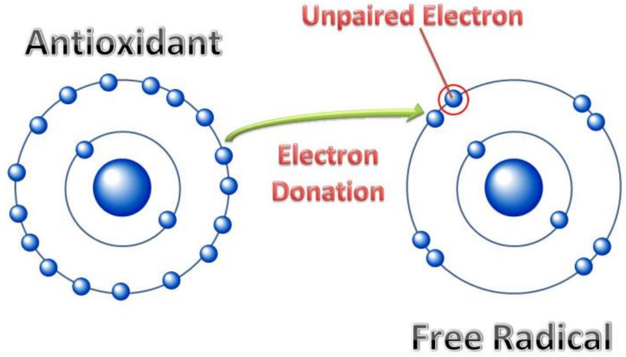
exposed outside

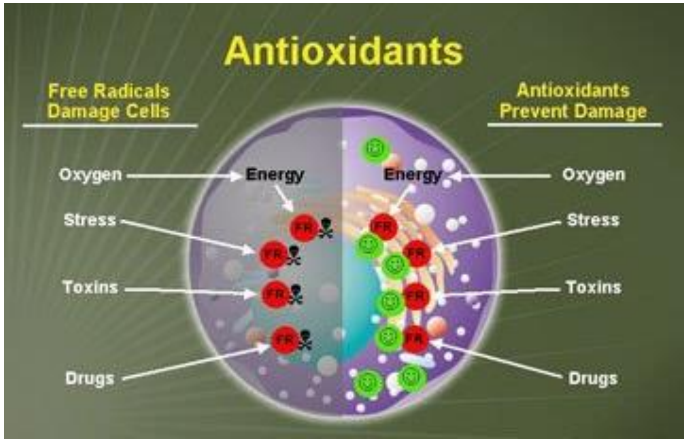
new

Antiradicals & Antioxidants

- Compounds that inhibit oxidation of molecules [1]
  - Donate electron to *free radicals*
- Prevent oxidative stress
- Food antioxidants
  - Fruits, vegetables, tea, coffee
  - Vitamin E, carotenoids (fat soluble)
  - Vitamin C (water soluble)
- Mechanisms[2]
  - Electron transfer
  - **Hydrogen atom transfer**

    POLYPHENOLS ACT via THIS MECAHNISM
  - Radical addition



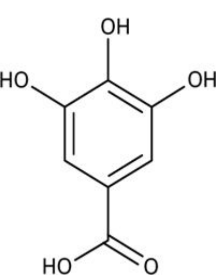


[1] Halliwell. *Annu. Rev. Nutr.* **16**, 33(1996).
[2] ValKo, Rhodes, Moncol, Izakovic, Manzul. *Chem-Biol. Inter.* **160**, 1(2006).

Phenolic antioxidants

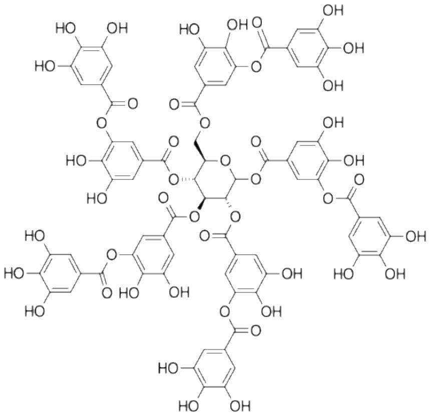
Antioxidant amount to reduce the radicals by 50% (lower = better)

Compound	EC_{50} (10^{-6} M)
Phenolic acids	
Gallic acid	5.1 ± 0.1
Protocatechuic acid	11.1 ± 0.0
Gentisic acid	7.6 ± 0.2
Siringic acid	12.3 ± 0.0
Caffeic acid	12.1 ± 0.2
Caftaric acid	20.4 ± 0.4
Other compounds	
Resveratrol	31.4 ± 0.3
Ascorbic acid	11.8 ± 0.2
Trolox	14.1 ± 0.0



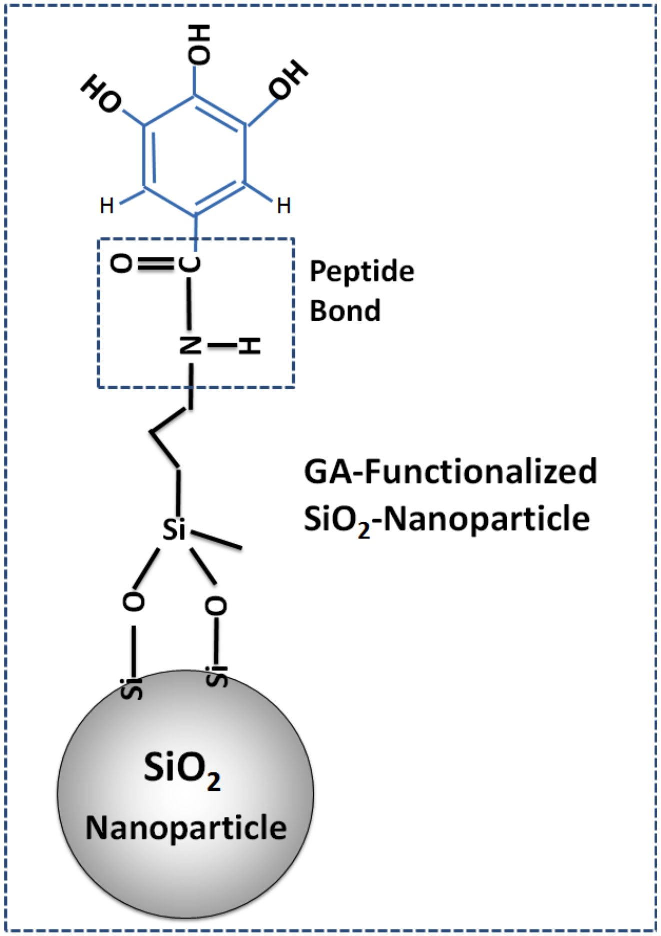
gallic acid

tannic acid

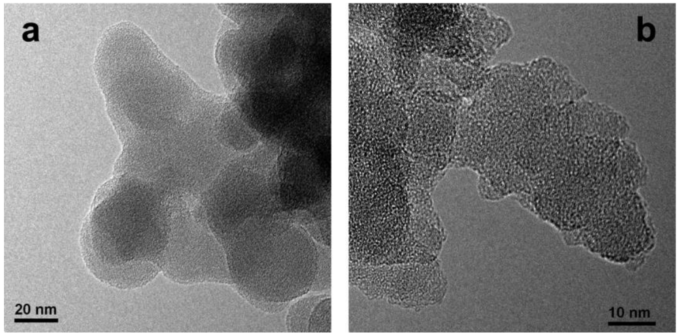


Villano, Fernandez-Pachon, Moya, Troncosco, Garcia-Parilla. *Talanta* **71**, 230 (2007).

Nanoantioxidant:Gallic acid grafting on SiO_2 nanoparticles



- Composition:
  - SiO_2 (FDA approved) – E551
  - Gallic acid (FDA approved)
    - E310 Propyl gallate
    - E311 Octyl gallate
    - E312 Dodecyl gallate
- Covalent grafting = stability
- Not compromising the –OH
  - responsible for antioxidant activity



pure SiO_2 functionalized SiO_2

Deligiannakis, Sotiriou, Pratsinis. *ACS Appl. Mater. Interfaces* **4**, 6609 (2012).

Antioxidant mechanism : Basics

Non-Radical

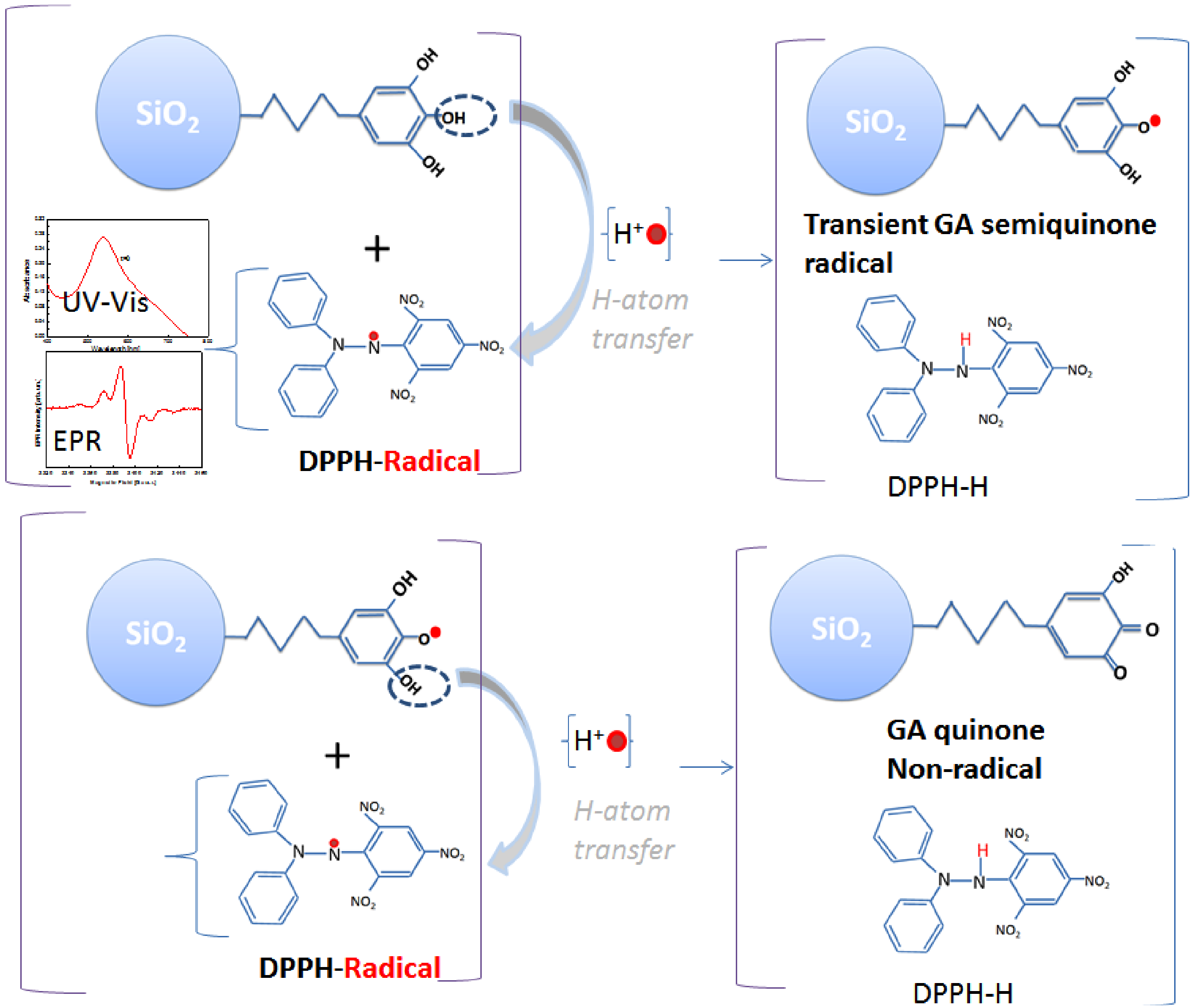
- **Electron transfer**
- [**E**lectron **D**onor] +**[Radical]**→**ED'** + **[Radical + (e-)]**

- **Hydrogen-Atom-Transfer**

[**R-OH**] +**[Radical]**→ **R-O'** + [**Radical + (e^-/H^+)]**

Non-Radical

Mechanism: Hydrogen atom transfer (HAT) in Polyphenols



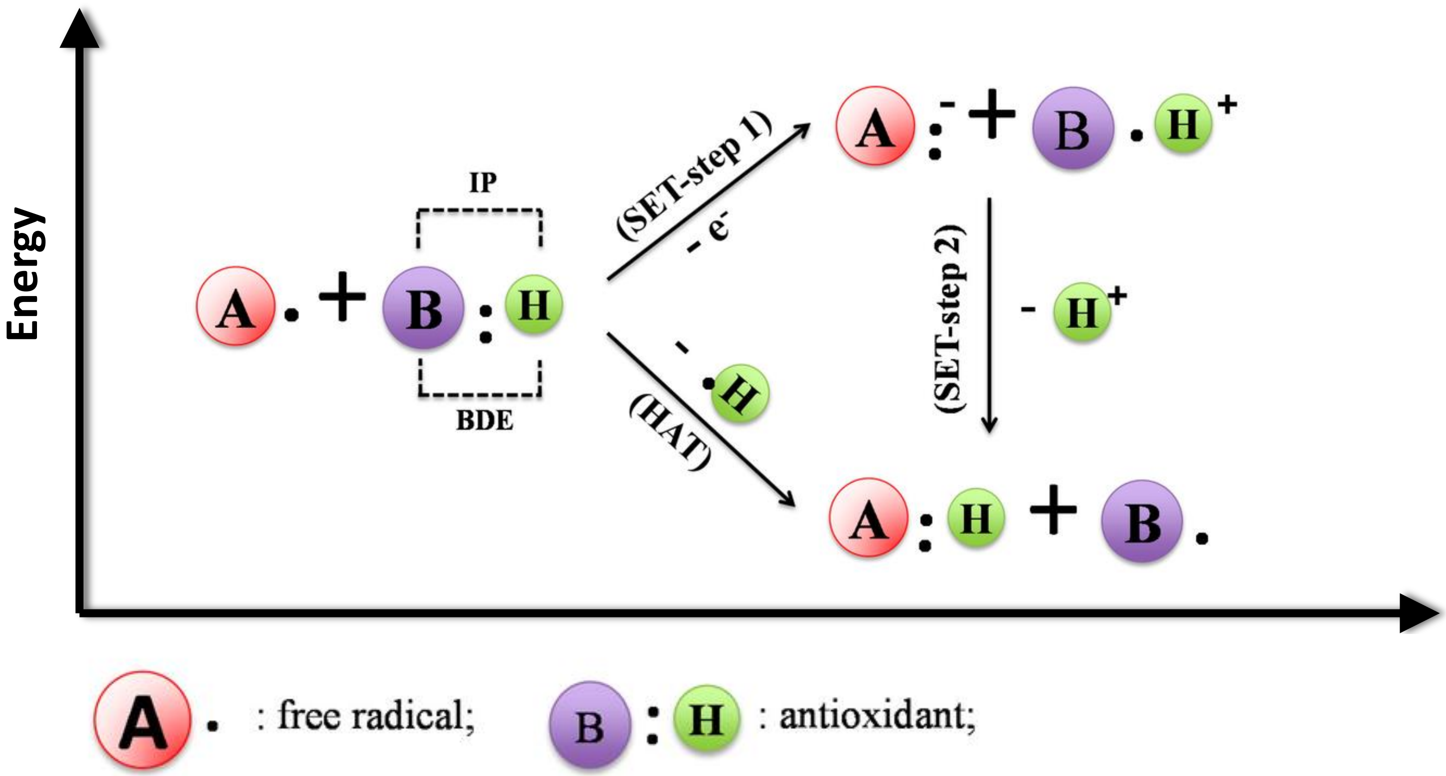
WHAT MAKES A "GOOD" ANTIOXIDANT ?

(............the energy cost of HAT)

Antioxidant HAT Energetics

HAT is energetically favored vs. ET

Hydrogen Atom = $[e^{-} + H^{+}]^{0}$ is a <u>**NEUTRAL**</u> entity



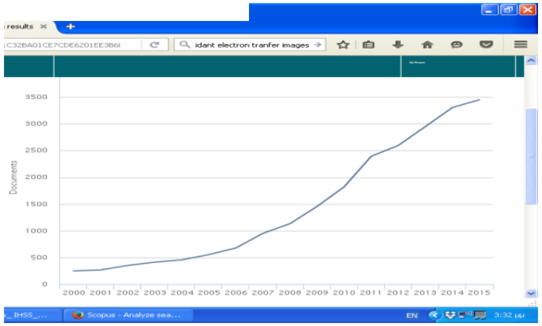
"IP" represents "ionization potential"; "BDE" represents "bond dissociation enthalpy"

Thus…

polyphenols are the stars of Antioxidants (strongly marketed already)

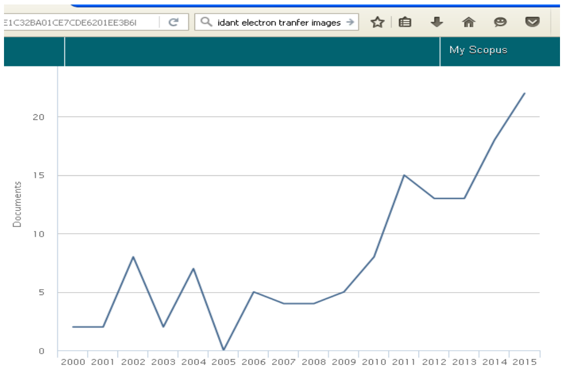
Humics….???????

SCOPUS-13-Sept-2016



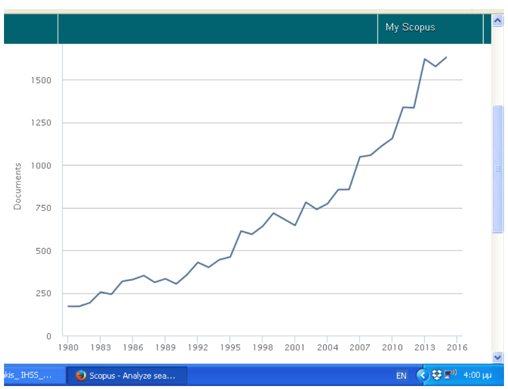
TITLE-ABS-KEY (**phenol AND antioxidant**)

36,692 document results



TITLE-ABS-KEY (**humic AND antioxidant**)

155 document results



TITLE-ABS-KEY (**humic**)

27,523 document results

TITLE-ABS-KEY (**hydrogen atom transfer**)

| 12,317 document results

TITLE-ABS-KEY

(**humic AND hydrogen atom transfer**)

1 document result

Journal of Colloid and Interface Science 455 (2015) 163–171

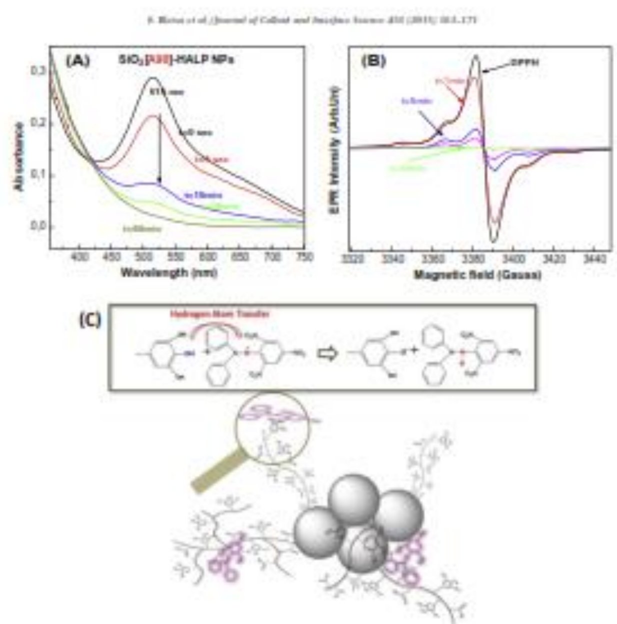
Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Interfacial Hydrogen Atom Transfer by nanohybrids based on Humic Acid Like Polycondensates

Eleni Bletsa [a], Panagiota Stathi [b], Konstantinos Dimos [c], Maria Louloudi [b], Yiannis Deligiannakis [a,*]



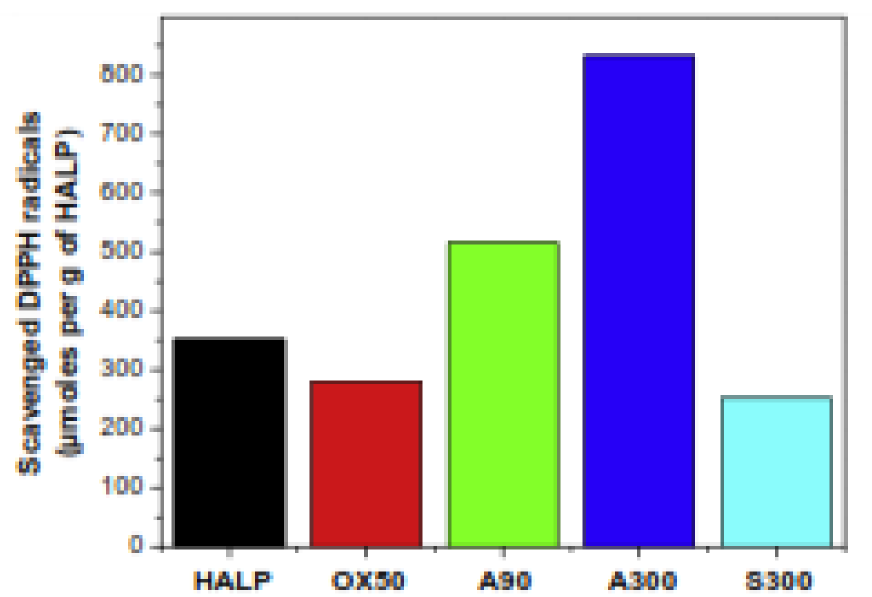
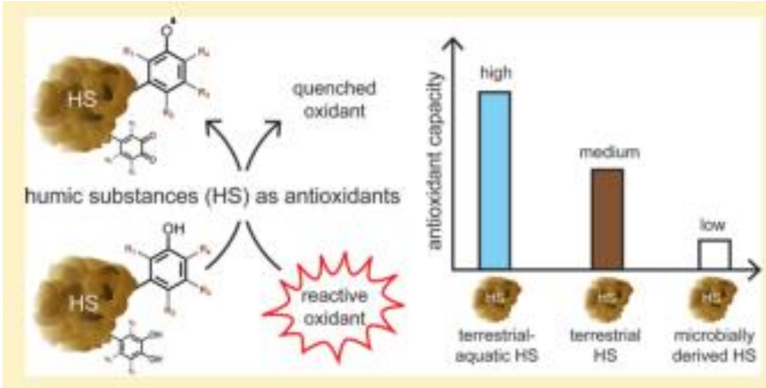
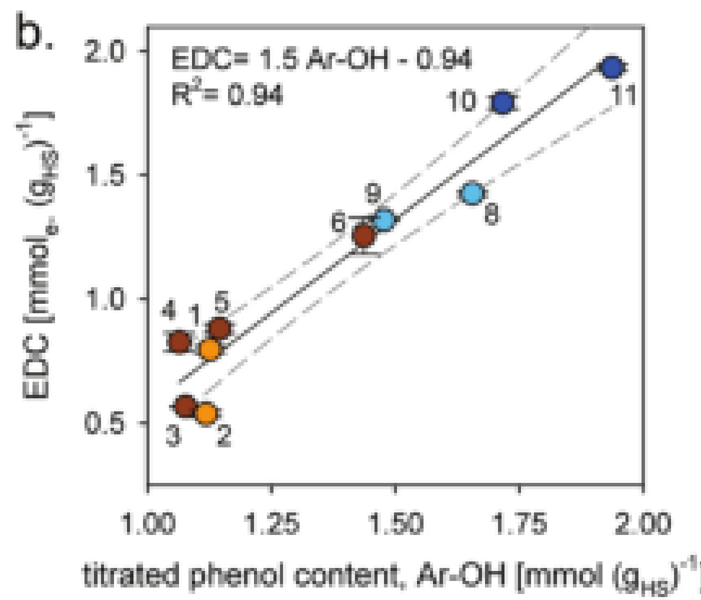
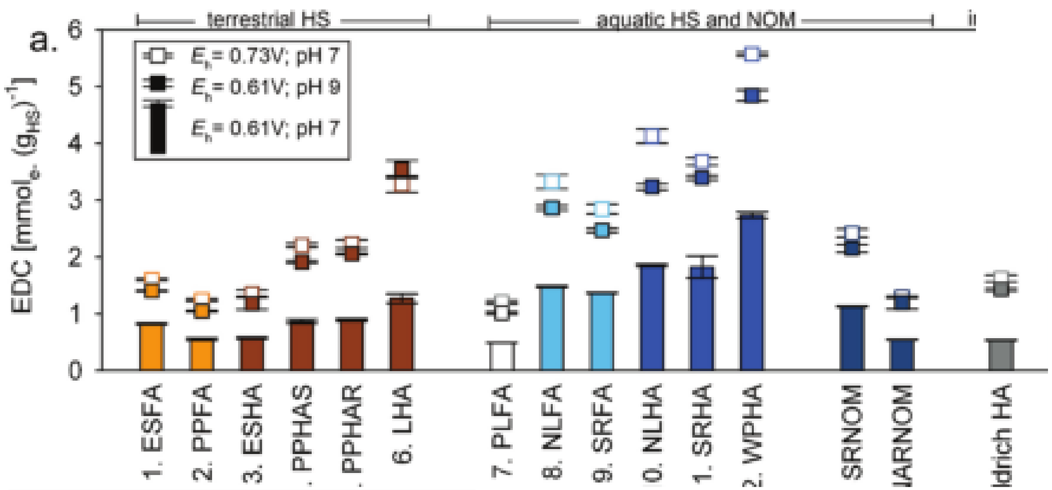
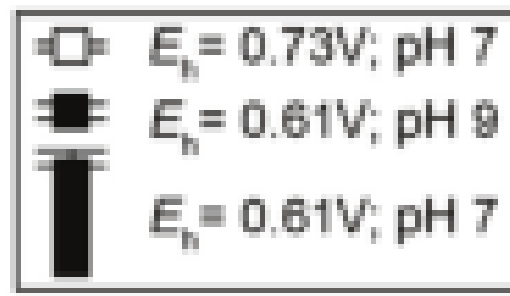


Fig. 9. The total DPPH radicals scavenged by the SiO_2–HALP NPs normalized per same mass of HALP.









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Antioxidant Properties of Humic Substances

Michael Aeschbacher, Cornelia Graf, René P. Schwarzenbach, and Michael Sander*

dx.doi.org/10.1021/es300039h | Environ. Sci. Technol. 2012, 46, 4916−4925

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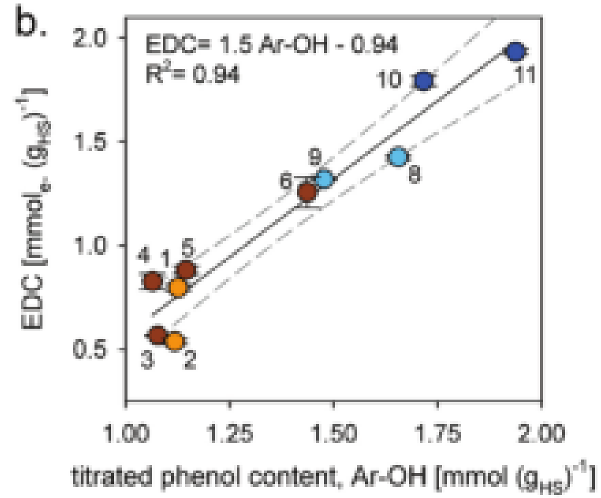


KEY RESULT (Sander/Schwarzenbach-2012)

UNDER STRONGLY OXIDIZING CONDITIONS (Eh>600mVolts)

Almost all (90%) of Humic Polyphenol–OH groups can donate 1electron to a STRONG OXIDANT

TRUE almost for ALL Humics tested so far.

At lower/more realistic Eh (-50 to +50mV) this drops by more than 10^4 times
1/10000 of OH are able to perform antioxidant ELECTRON TRANSFER

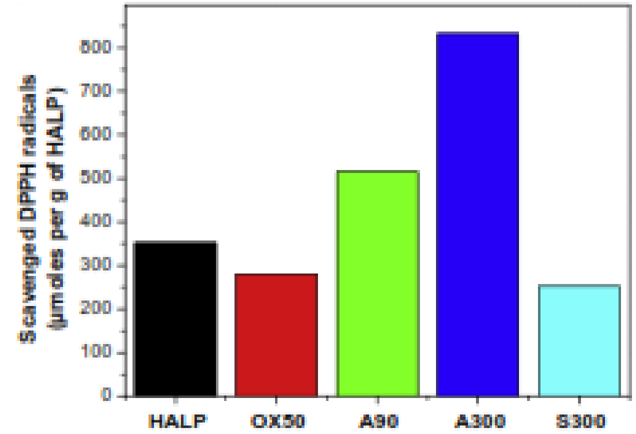


Fig. 9. The total DPPH radicals scavenged by the SiO_2–HALP NPs normalized per same mass of HALP.

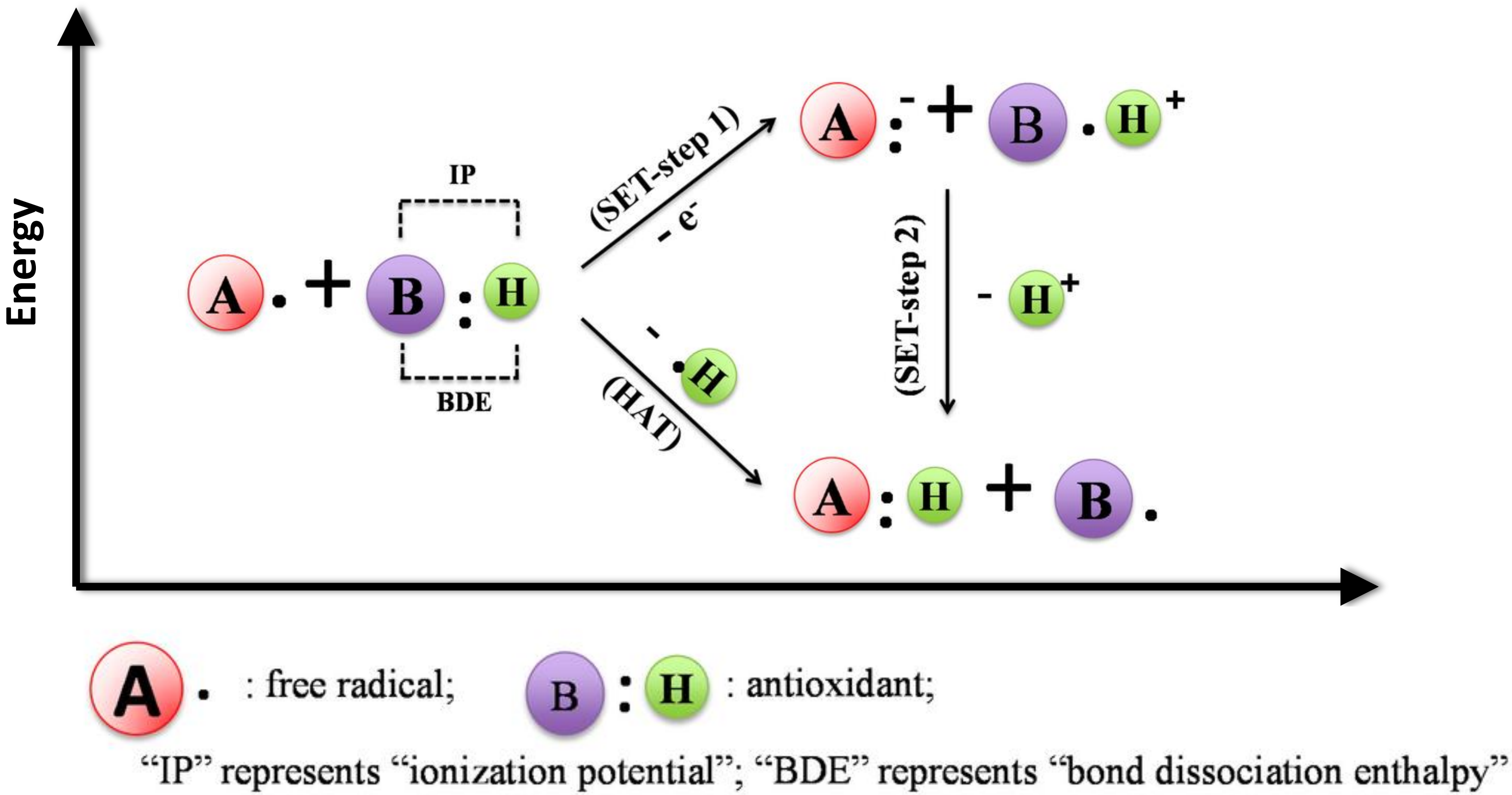
KEY RESULT (Bletsa/Deligiannakis 2015)

UNDER REAL OXIDIZING CONDITIONS (Eh -50/+50mVols)

[a] 20% of Humic Polyphenol–OH groups can perform HAT

[b] This antioxidant activity is improved by 300%
when HA is attached on an appropriate SiO_2 nanoparticle

HAT is energetically favored vs. ET



AIM

IS TIME TO BUILD

A FIRM PHYSICOCHEMICAL BASIS

of Antioxidant Hydrogen Atom Transfer
by Humic Acids and HA@SiO_2 Nanohybrids

Then …

Discuss it vs.

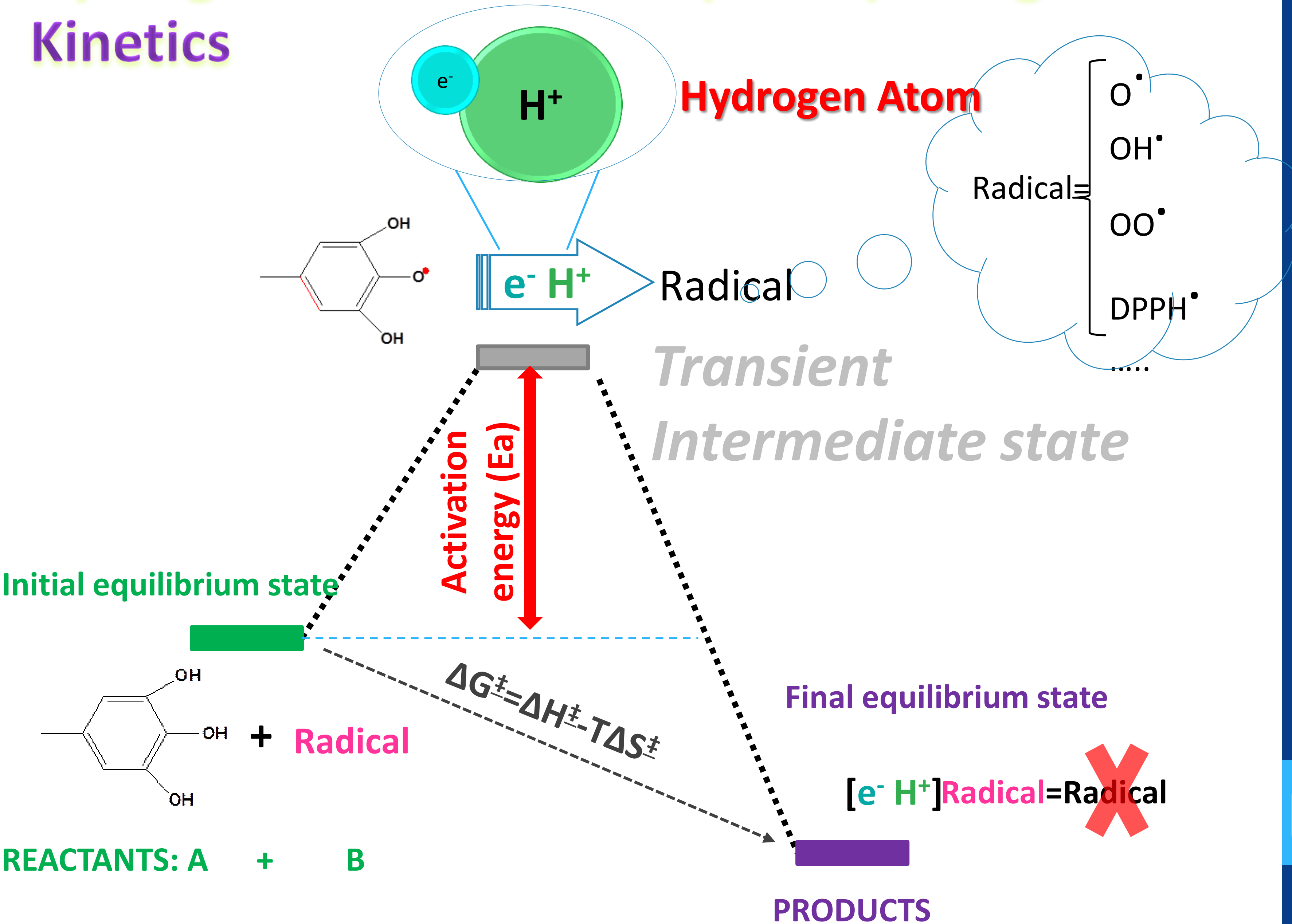
H^+ binding

Stable Radicals

Polyphenol Concentration

our
Thermodynamic Context

Hydrogen- Atom-Transfer (H.A.T.) Energetics-Kinetics



RESULTS

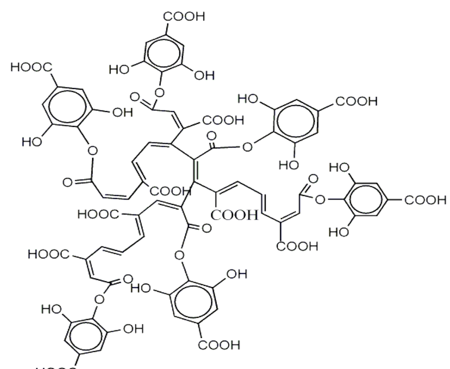
Nanoparticles + Humics → SiO_2@HA Nanohybrids

SiO_2 Nanoparticles

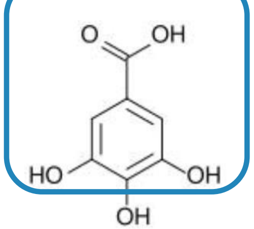
SSA=$50 m^2/g$
R=40nm

[1] NATURAL Lignite HA
Well characterized Lignite HA
(~IHSS Leonardite HA LH4)
Drosos, Deligiannaksi JCIS 2009

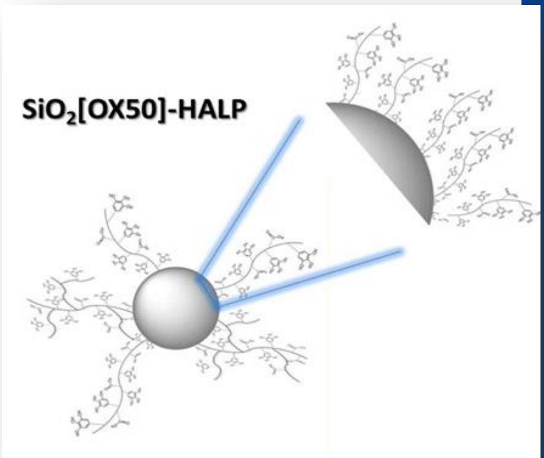
[2] Humic Acid Like Polycondensate (HALP)
Well characterised model
Giannakopoulos Coll. Surf.-A 2010)



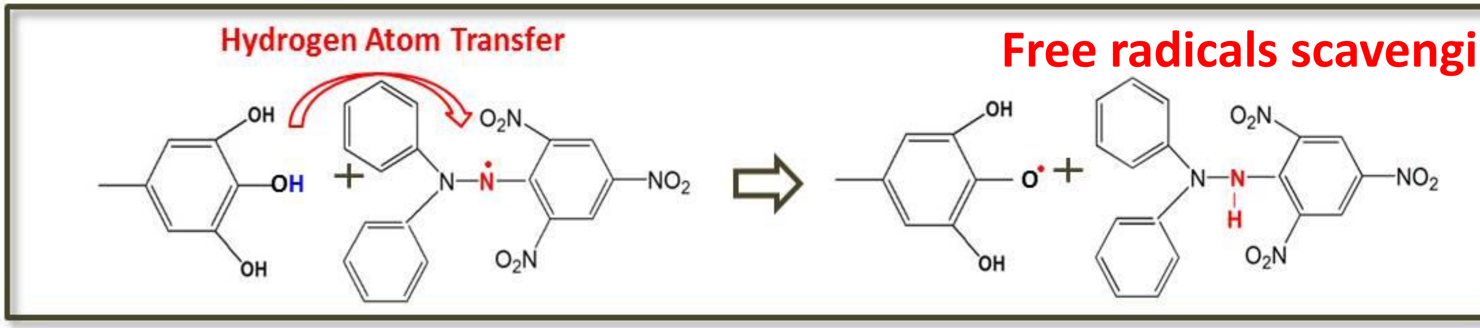
[3] GA-monomer

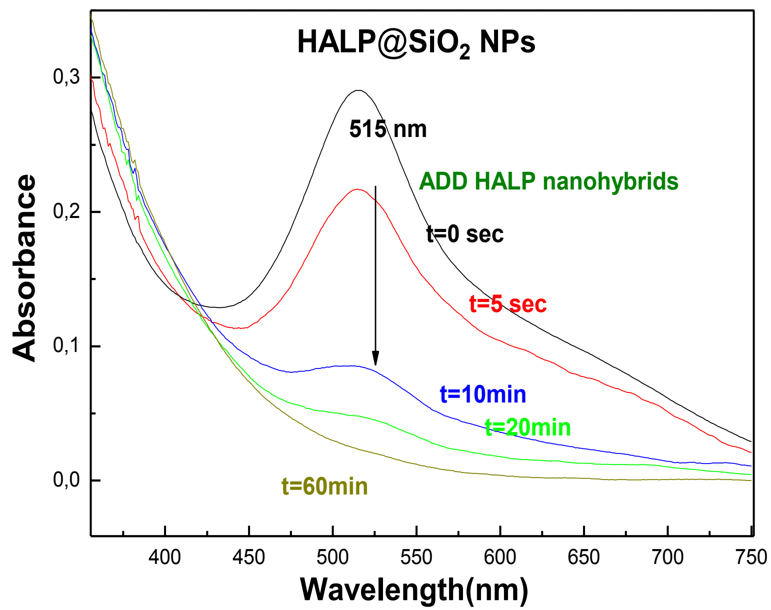


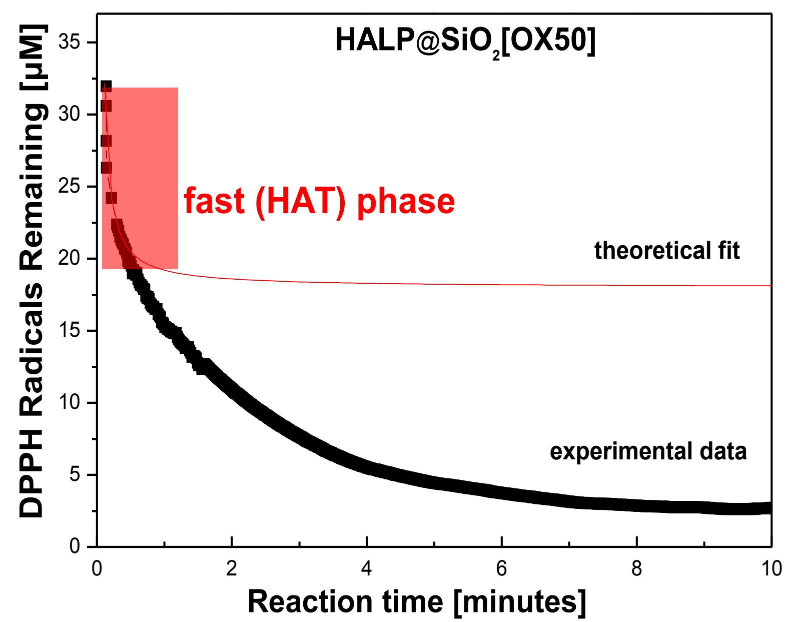
Covalent immobilization



Evaluation of Hydrogen Atom Transfer for HALP-nanohybrids by the DPPH˙ method







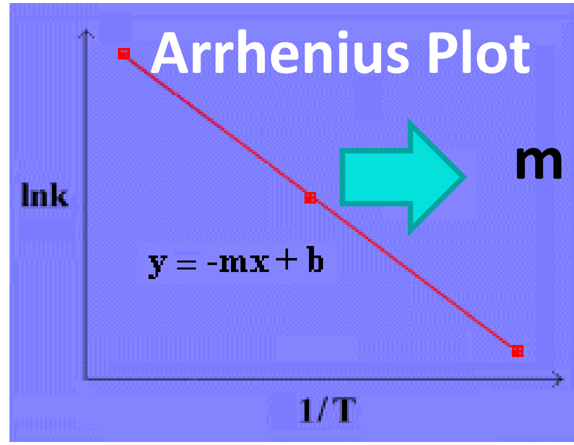
E. Bletsa, P. Stathi, K. Dimos, M. Louloudi, Y. Deligiannakis, Journal of Colloid and Interface Science 455 (2015) 163.
Y. Deligiannakis, G.A. Sotiriou, S.E. Pratsinis, Acs Applied Materials & Interfaces 4 (2012) 6609.
▪Brand-Williams, W.; Cuvelier, M. E.; Berset, C. LWT-Food Sci. Technol. 1995, 28, 25–30
▪Mishra, K.; Ojha, H.; Chaudhury, N.K. Food Chem. 2012, 130, 1036–1043

Thermodynamic analysis 1min

Arrhenius equation → ACTIVATION ENERGY(Ea)

Activation Energy (Ea)

$$k = A * e^{-\frac{Ea}{RT}}$$



$m = - E_a / R$

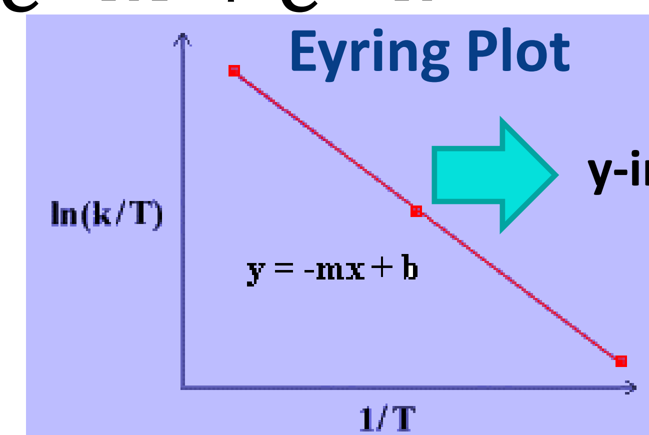
Eyring-Polanyi equation

Enthalpy of activation

$$k = \frac{k_B * T}{h} * e^{-\frac{\Delta H}{RT}} * e^{\frac{\Delta S}{R}}$$

Entropy of activation

Reaction rate



$-\Delta H/R$

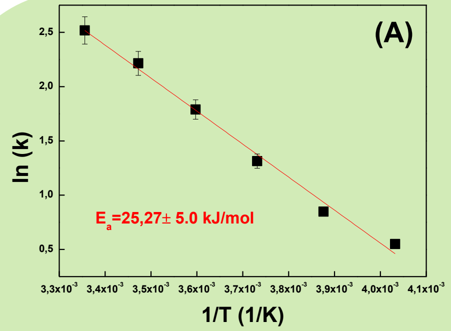
y-intercept: $\Delta S/R + \ln kB_h$

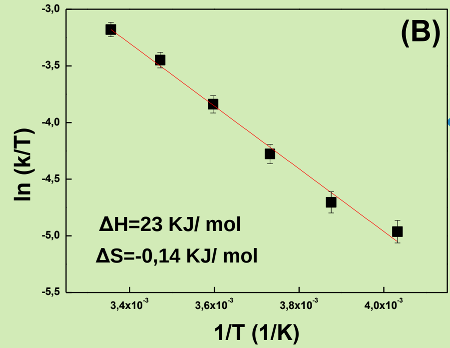
Thermodynamic analysis

HALP nanohybrids

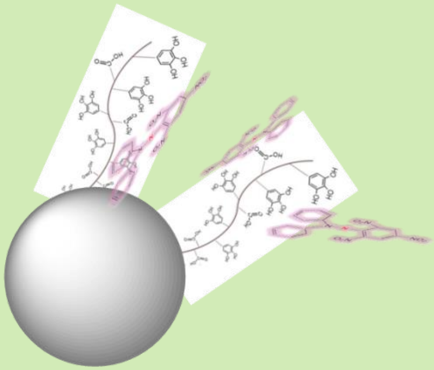
Arrhenius Plots

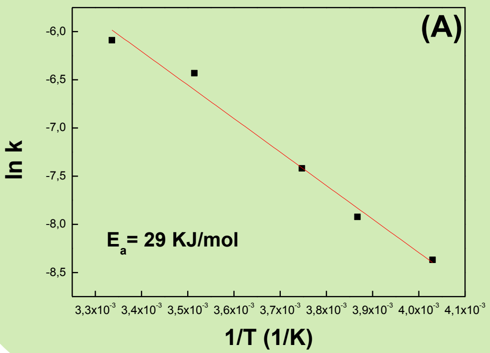
Eyring-Polanyi Plots

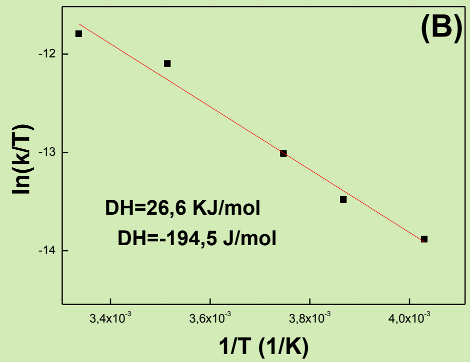




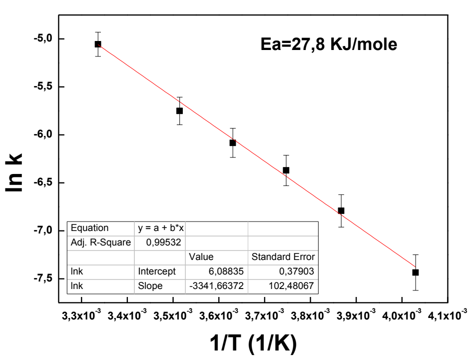
HALP@SiO$_2$[50]+DPPH$^{\bullet}$

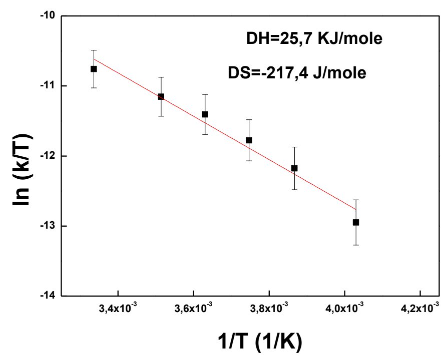




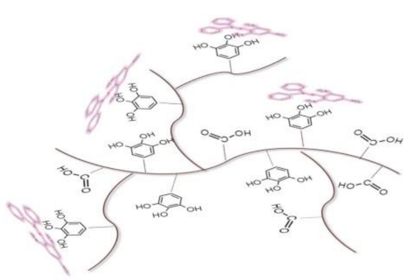


HALP@SiO$_2$[300]+DPPH$^{\bullet}$





HALP+DPPH$^{\bullet}$



Thermodynamic comparison of H-Atom transfer between LHA, HALP, GA & their nanohybrids

Sample	Ea(kJ/mol)	ΔH‡ (kJ/mol)	ΔS‡ (kJ/mol)	ΔG‡(kJ/mol)
Ligntite HA (LHA)	+28.0±0.5	+25.7±0.5	-0.218±0.02	+90.9±1
LHA@SiO_2[50]	+11.8±0.5	+9.5±0.5	-0.27±0.03	+90.5±1
HALP	+27.8±0.2	+24.8±0.3	-0.21±0.03	+88.3±1
HALP@SiO_2[50]	+23.8±0.5	+21.4±0.3	-0.22±0.03	+87.4±1
GA	+35.7±0.2	+33.5±0.2	-0.187±0.03	+89.6±1
GA@SiO_2[50]	+23.2±1	+20.1±1	-0.174±0.05	+72.9±1

Ea→LHA~HALP<GA (HAs are better than monomeric GA)

Ea→ HA@SiO2<HA in solution (SiO_2=no effect on HA.

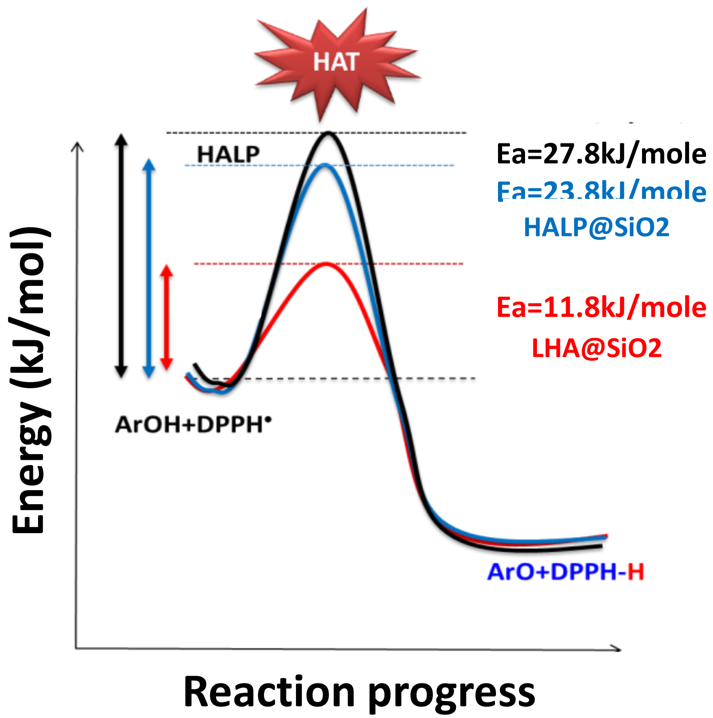
Ea→ LHA@SiO2<HALP@SiO2 in solution (HA ‘macrostucture‘ determines Ea)

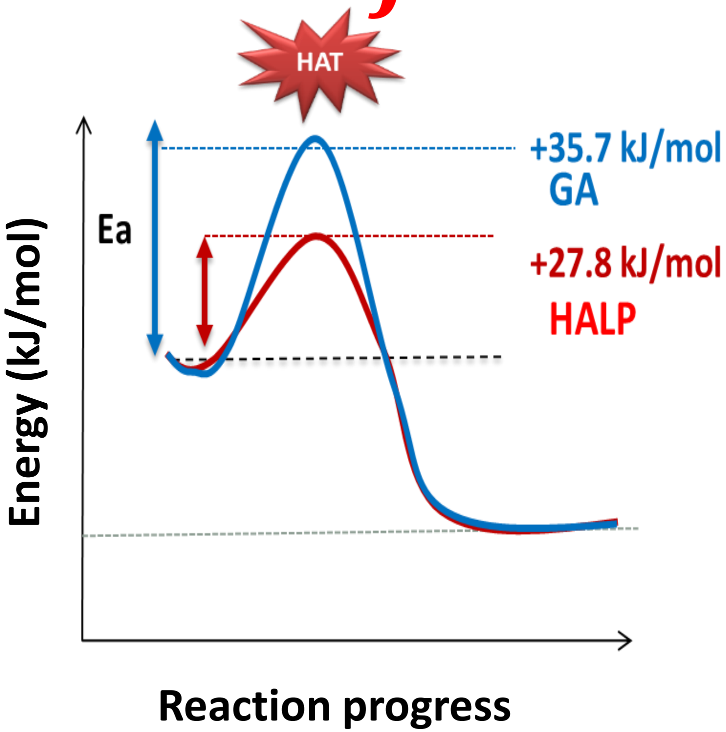
Ea→ GA@SiO2<GA in solution

Ea→ GA@SiO_2 NPs~HALP@SiO_2 NPs

Dispersion of polyphenolic molecules onSiO_2 NPs:→ lowers Ea

Key RESULT-1: ENERGETICS of Antioxidant H-Atom Tranfer

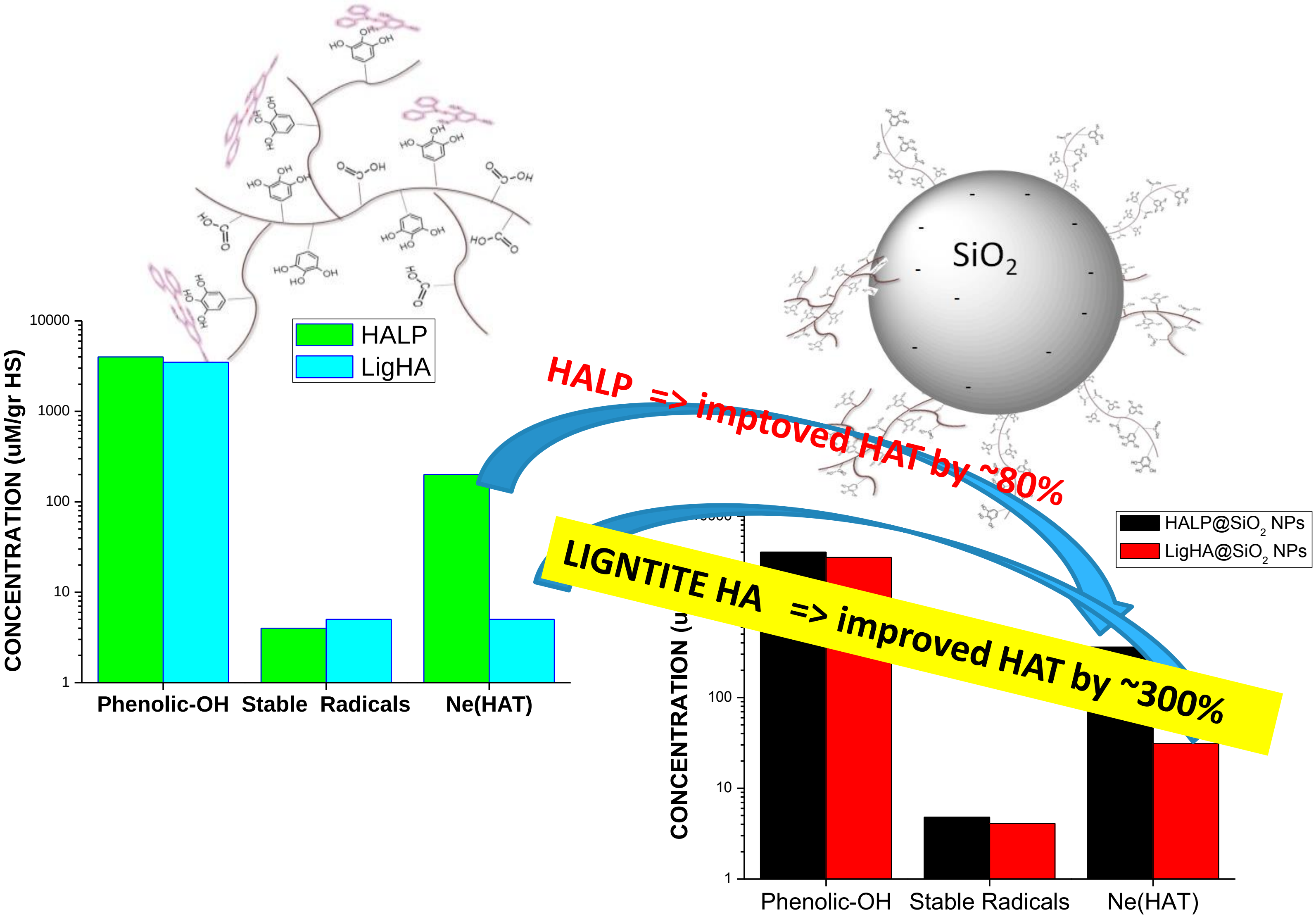




- ✓ HAs are more efficient than GA monomer in Antioxidant HAT (lower Ea by ~ 17 kJ/mole)
- ✓ SiO_2 particles lower (Ea) on HAT performance by HA monomer
- ✓ HA-local network determines the Energetics of HAT/ it lowers Ea

Key Result-2

HA-NanoHybrids : better Antioxidant-HAT than homogeneous HA

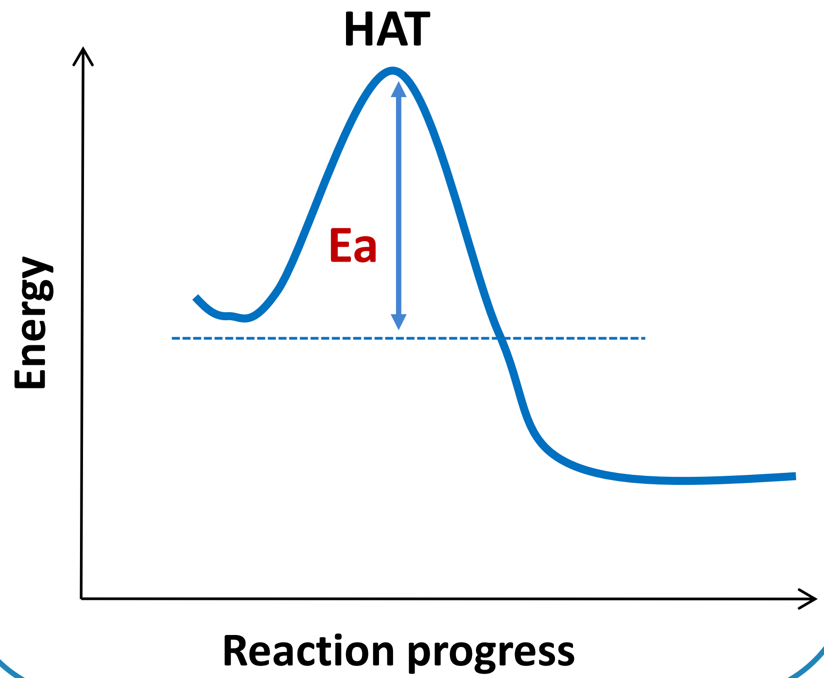


Bletsa, E; Louloudi, M; Deligiannakis Y.; Envriron. Sci. Technol. 2017 (submitted)

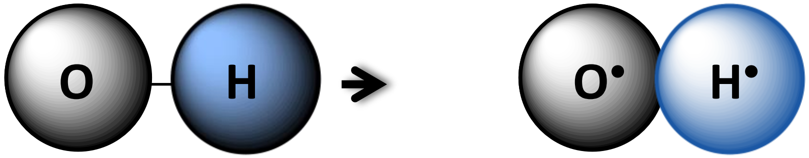
Key Result-3

HA-NanoHybrids : Thermodynamics vs. chemical information

$$ArOH + DPPH^{\bullet} \rightarrow ArO^{\bullet} + DPPH{-}H$$



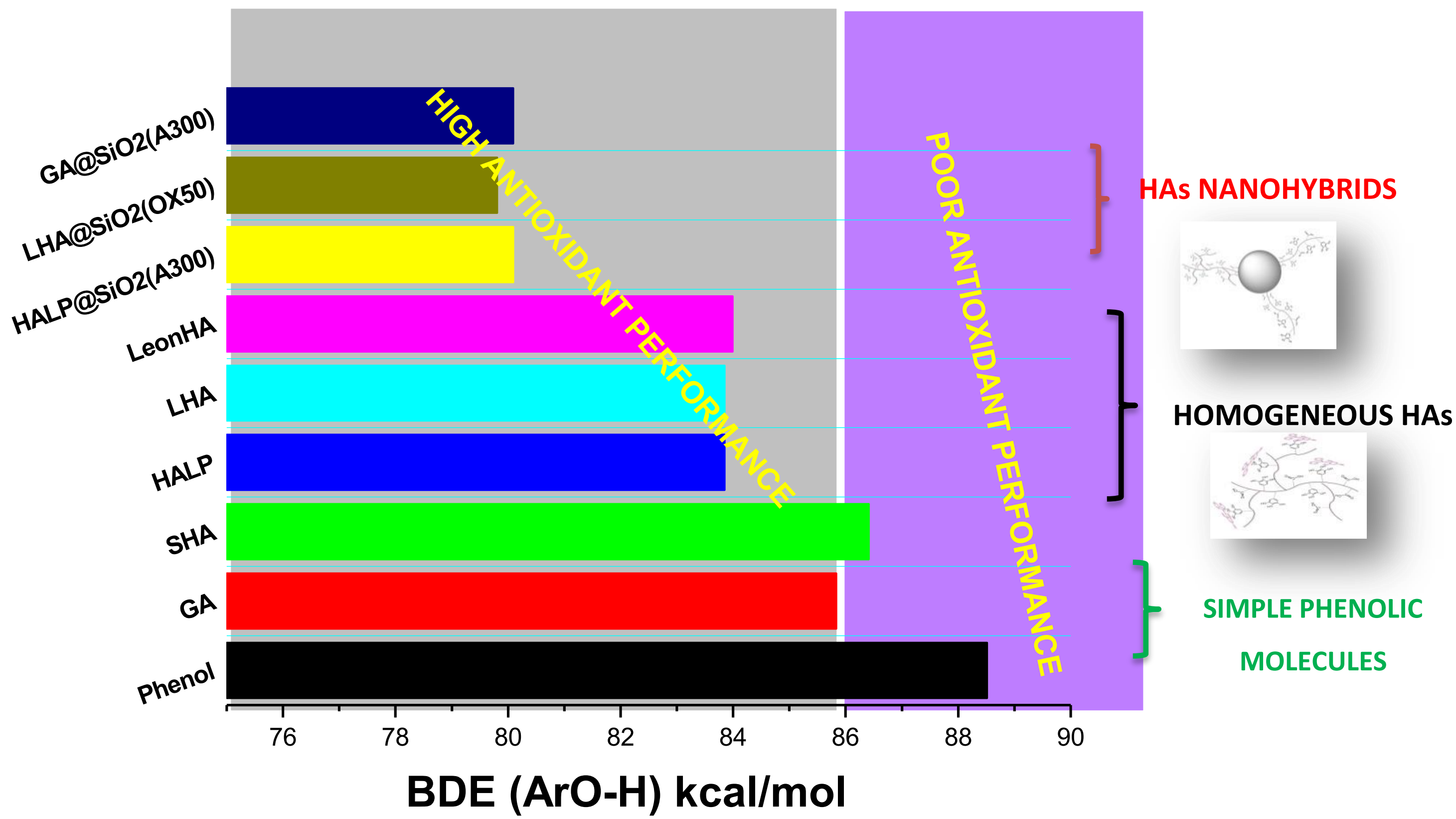
Bond Dissociation Enthalpy (O-H)



Ea(kcal/mol)=0.918 BDE-70.27

Bond Dissociation Enthalpy (BDE kcal/mole)=(Ea+70.27)/0.918

Foti M., J. Org. Chem. ***2008,*** *73, 9270-9282*



✓Immobilization of HAs on SiO2 particles→ lower BDE (O-H)→ better HAT performance by HA-nanohybrids

New Technology Oportunities in HumicsTechnology

Humic Acid-Nanoparticle-NanoHybrids

More efficient than solution-phase Humics *(Bletsa et al Env. Sci. Techn. 2017)*

[antioxidant (this work + Bletsa et all JCIS 2015 +D eligiannakis et al ACS App Mat Interfaces 2012]

+EU Patent

[metal removal from waters, filtering/ Stathi, Deligiananakis JCIS 2012]

For industrial applications...of HA-Nanohybrids. NEED for a LOW-COST HIGH-STANDARDISATION PROCESS FOR Nanoparticle production

REUSABLE

(we can recover HA@SiO_2 by a simple spinning and reuse it)

EASY TO HANDLE

(process engineering at industrial scale)

+++++ OTHER OPORTUNITIES: *Humic Acid-NanoHybrids*

--CONTROLLED RELEASE OF NUTRIENTS,

---TRACERS IN “HA-technological “ products

SCALE UP PRODUCTION ON NANOMATERIALS\
by
FLAME SPRAY PYROLUSIS-TECHNOLOGY
CONNECTING LAB-TO-INDUSTRY

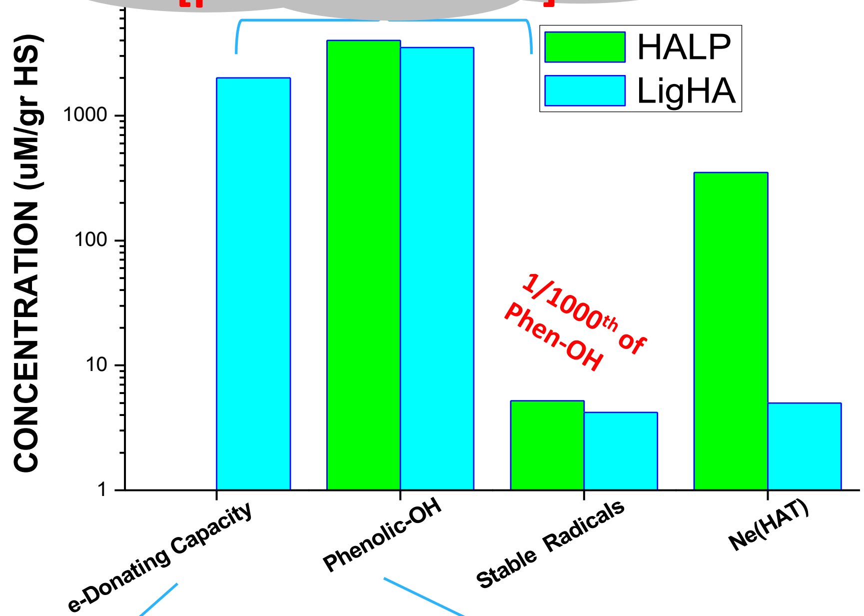
Nanotechnology Flame Spray Pyrolysis at the Lab of Physical Chemistry of Materials & Environment at Univ. of Ioannina

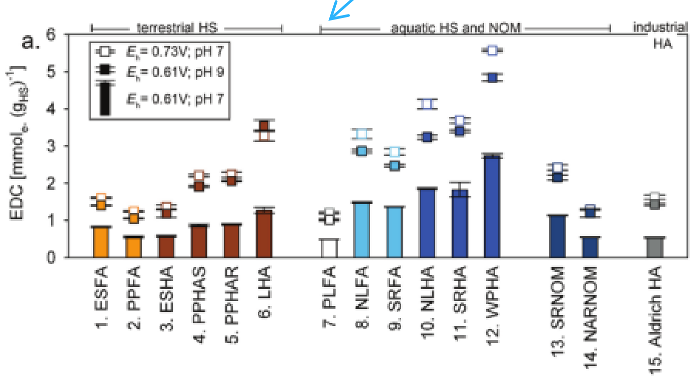
Flame **S**pray **P**yrolysis Lab at UoI

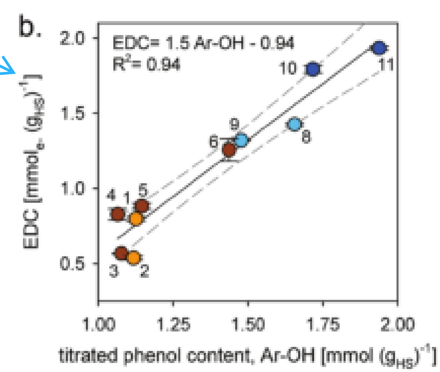
Key Result-3

Arithmetics of functional moieties: R-OH, e-donation, stable Radicals, HAT

e-donation capacity
~[phenol content]







SUMMARY-I (on the performance of Natural Lignite HA in solution phase)

[1] Humic Acids' -Phenolic OH => Efficient in Antioxidant HAT

Lower activation energy in HA than in GA monomers

Kinetic limitations are imposed by Diffusion from solution into the HA-polymer

(NOT by Hydrogen Atom Tranfser -this is FAVORED in HA)

[2] Natural Lignite HA : severe inhibition of the NUMBER of active HAT-performing R-OH (280umole by HALP, 5um/gram by LHA)

[3] Activation energy of these active R-OH is the SAME in Natural HA as in synthetic HALP

CONCLUSION-1

SAME HAT MECHANISM (therrmodynamics) in LHA , HALP and monomeric GA

NUMER of active R-OH sites (to perform antioxidant HAT) are 100-fold less available in LHA than in HALP.

SUMMARY-II (on the performance SiO2@HA vs. Solution phase HA)

[1] SiO2@HA hybrids have improved Antioxidant efficiency

[2] Ligntite HA has 300% improved Antioxidant efficiency

This is not because of change in the HAT energy cost (Ea is the same in HALP, LHA, SiO2@HALP, SiO2@LHA)

CONLUSION-2

The better performance of SiO2@HA hybrids is
Better arrangement of the HA-macromoleculs on the SiO_2 particles thus lowering diffusion barriers.

Other factors……: a fraction of R-OH in LHA is "oc luded" in LHA domains with low accessibiltiy by solvent dynamics (to be tested by screening various soil/ligtnie HAs

CONCLUSION-Challenges

Antioxidant HAT at physiological conditions can be accurately evaluated by the DPPH protocol.

Natural HAs of different compositions have comparable [R-OH] (1-4 mmol/gram), however the Antioxidant R-OH fraction will be determined by the local macrostructural profile of each HA (to be screened –tabulated in soil vs. aquatic vs. lignite vs. pyrolysed carbons etc)

HA-Nano composites offer novel technology/research oportunities.

Thus, the FIRM structural/functional data accumulated -so far- by the rerearchers working on HA science (guided by IHSS and HA-research pioneers) CAN be capitalised in competitive technology-oriented applications.

ICCE 2017
OSLO

Thank you!!!

IHSS
NATURAL ORGANIC MATTER RESEARCH