Efficient synthesis of DHA transition metal chelates as potent antioxidants, enzyme inhibitor and antimicrobial agents.

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Abstract: A large *in vitro* biological screening and an efficient with easy access to a family of transition metal complexes of dehydroacetic acid (**DHA**) are reported. The obtained complexes (**1-4**) with some transition metal of interest: **Ni (II), Co (II), Zn (II), Mn (II)** respectively, were fully characterized by MP, UV-Vis and FT-IR spectroscopy; several *in vitro* biological tests were performed on this series of compounds to explore its therapeutically potential in order to continue further investigations and exploring it as new target drugs. In this case, enzymatic activity: as urease inhibitors and antioxidant activities: ABTS scavenging activity, β-carotène linoleic acid bleaching activity, Ferrous ions binding effect, Copper (CCA) and ferrous chelating activity, gave good values of IC50 for all studied complexes **1-4** in range of 8,20 ±0,39-10,62 ±0,01 μg/mL for urease inhibiting test better than DHA and used standard Thiourea (IC50=11,57±0,68 μg/mL), interesting results are also obtained for compound **2** in ability of chelating ferrous ions with an IC50=14,53±0,92 μg/mL, comparing with tested standard EDTA (CI50=8,80±0,47 μg/mL), for all cited applications complex **4** is mostly a hit, while antimicrobial activity gave better results with free ligand DHA, discussion on molecular structure and predicted SAR will be given.
Keywords: DHA, Transition metal complexes, Biological activities.

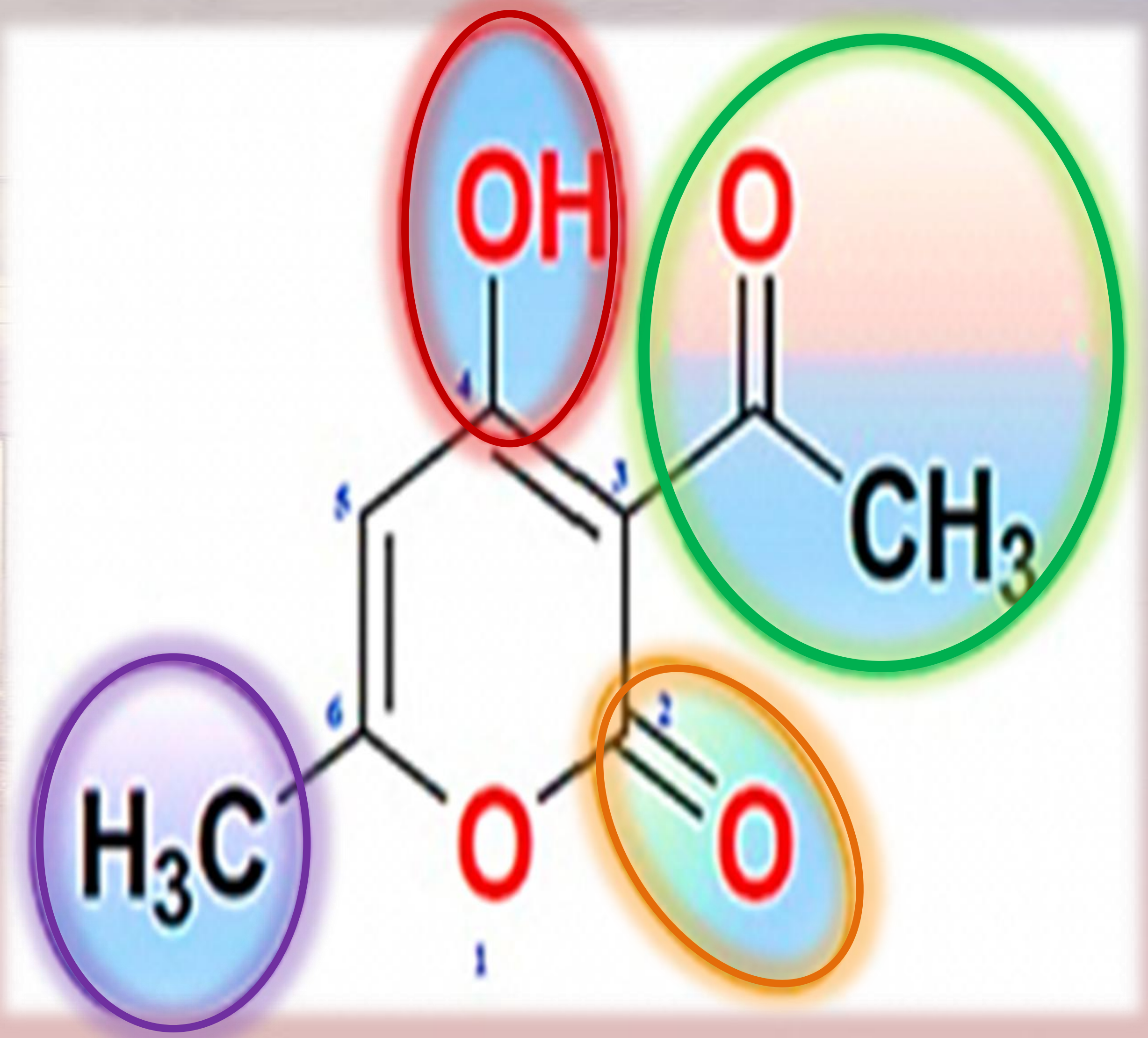
Déhydroacétic acid

DHA or 3-acetyl-4-hydroxy-6-methyl-2-pyrone)

Identified structure in **1924 by Rassweiler et Adams.**

Several active centers:

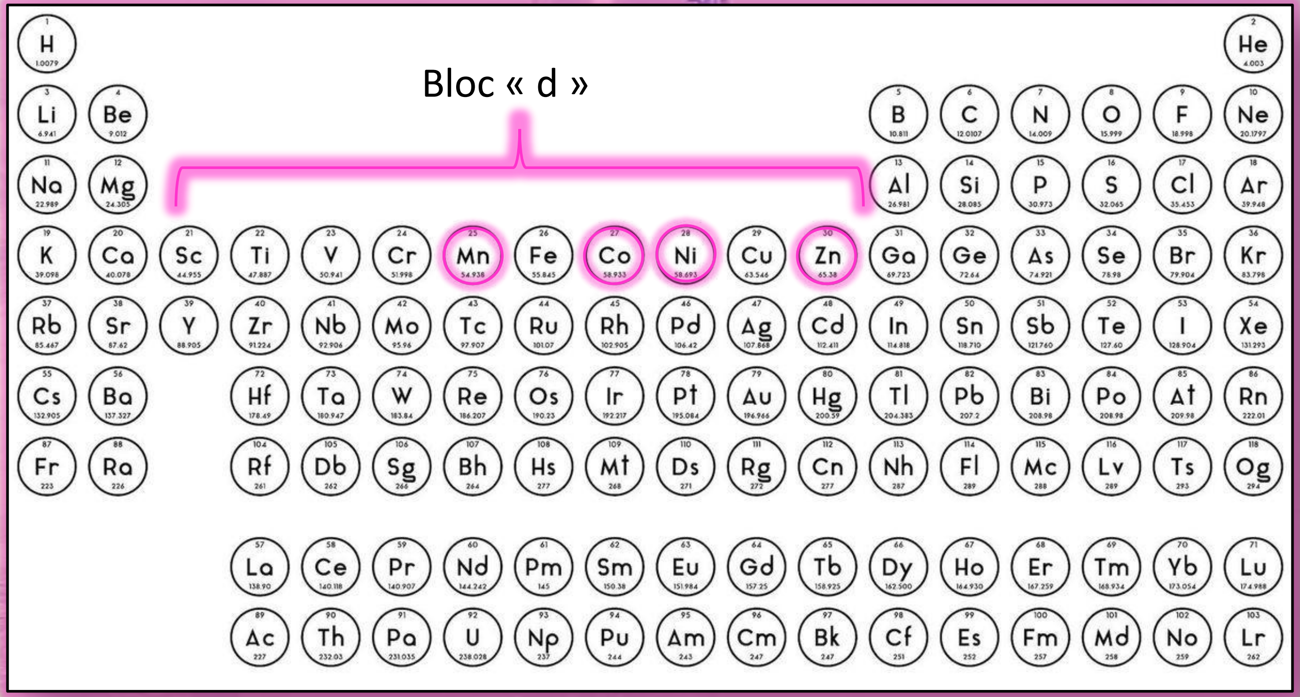
- Position C3.
- Positions C2, C4 et C6.
- Position C5.



Transition Metals

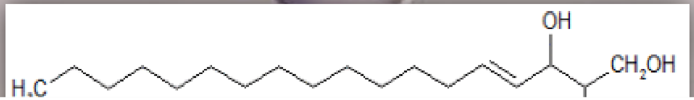
Bloc «d».

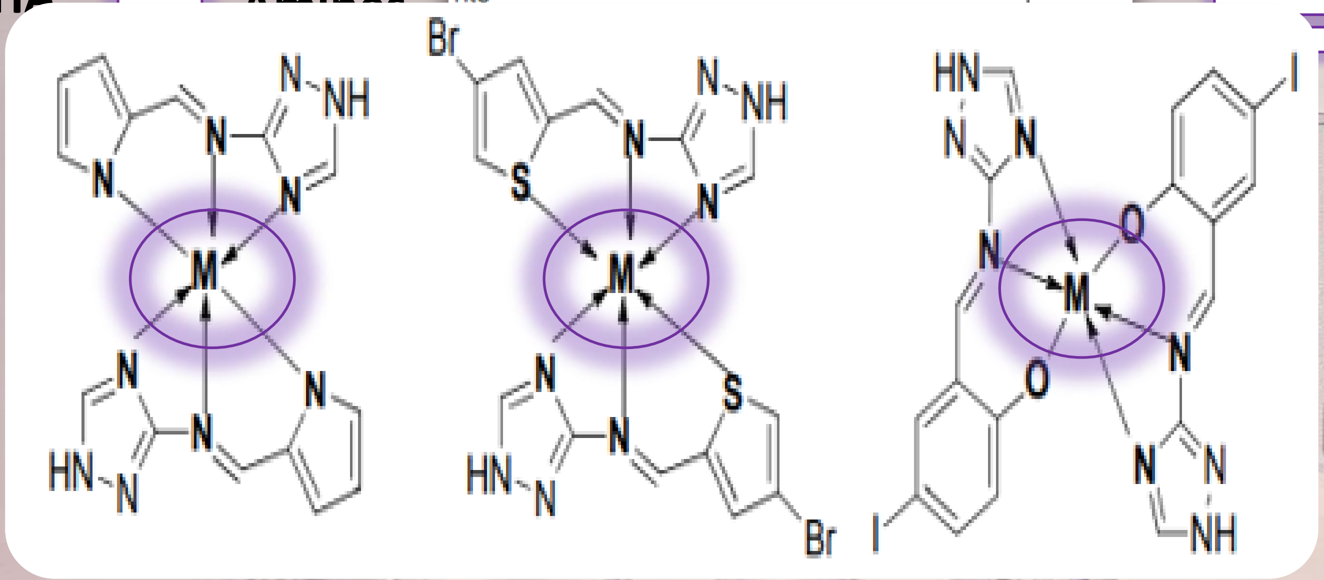
Metal	Electrons de valence de la couche « d »
Nickel	**8 électrons**
Cobalt	**7 électrons**
Zinc	**10 électrons**
Manganèse	**5 électrons**

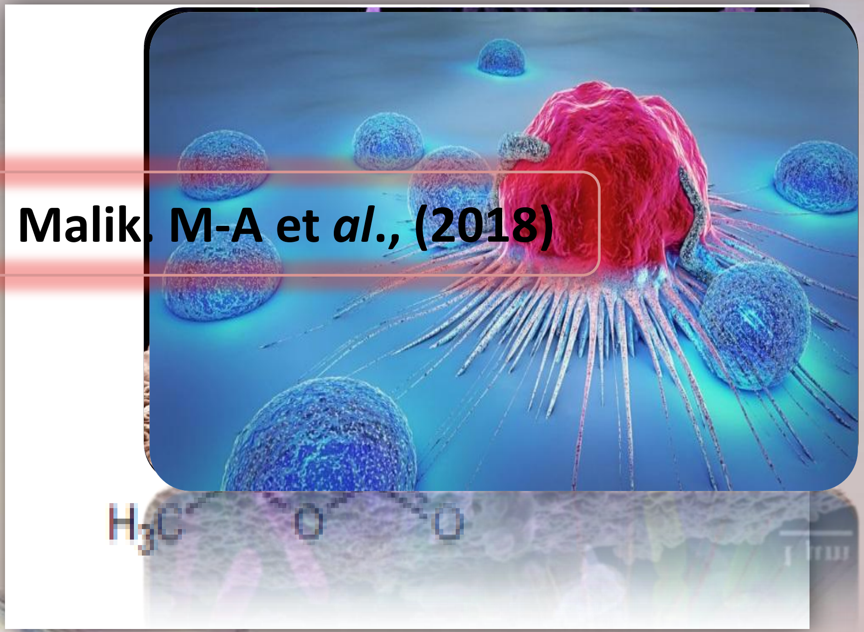


Biologiocal activities of DHA and dérivatives

DHA + Amines







Malik, M-A et *al*., (2018)

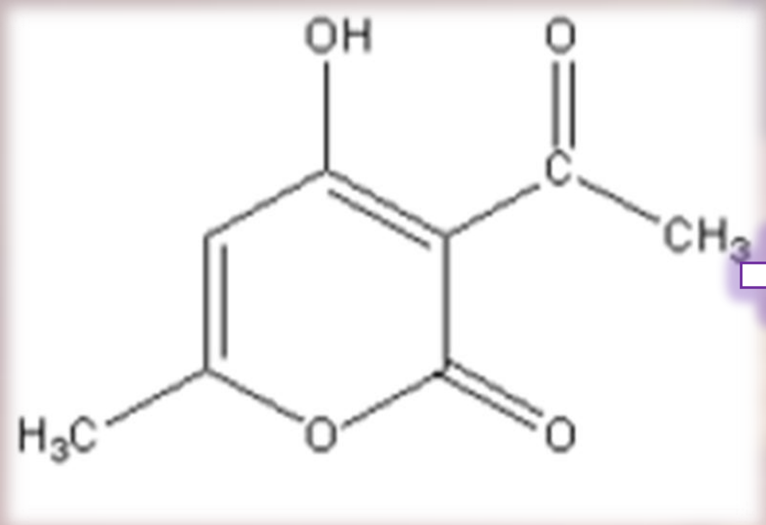
Maiti, (1998)

M = Ni
Co
Mn
Zn

Ullah et *al*., (2012)

Biological activity

Synthesis of DHA chelates



+ MCl_2, xacetone

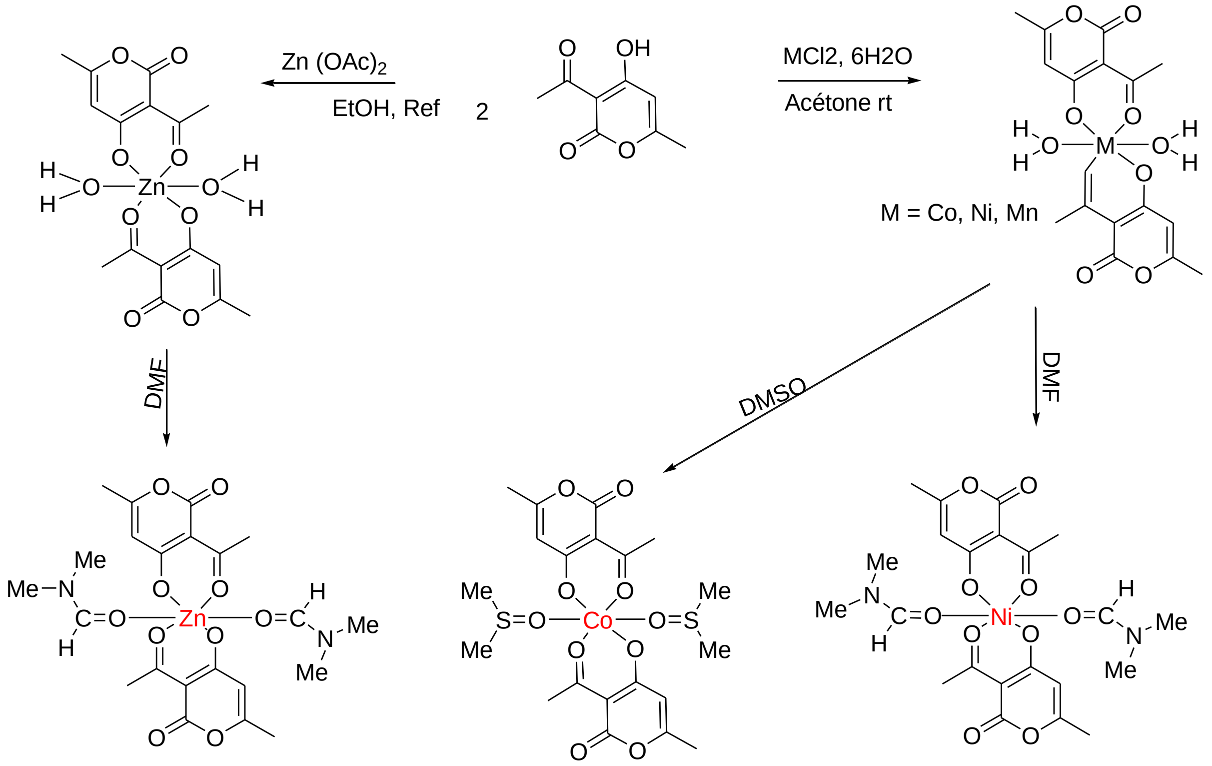
CH_3COONa

EtOH

M= Ni, Zn, Co, Mn

Chelates	Color	Yield%
$Co(DHA)_2.2H_2O$	Rose	81
$Ni (DHA)_2.2H_2O$	Vert	72
$Zn(DHA)_2.2H_2O$	Blanc	91
$Mn(DHA)_2.2H_2O$	Jaune	43

Syhthesis pathways



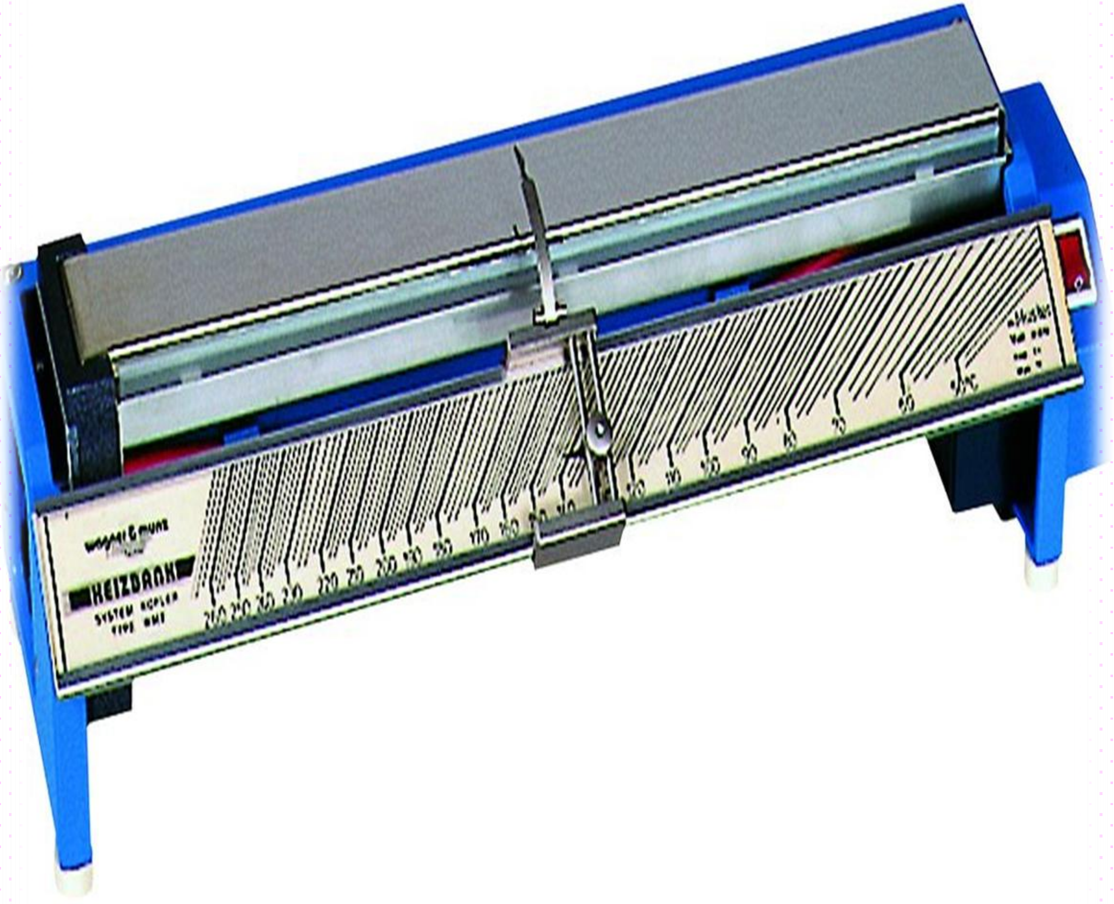
Spectroscopic identification

Melting Point

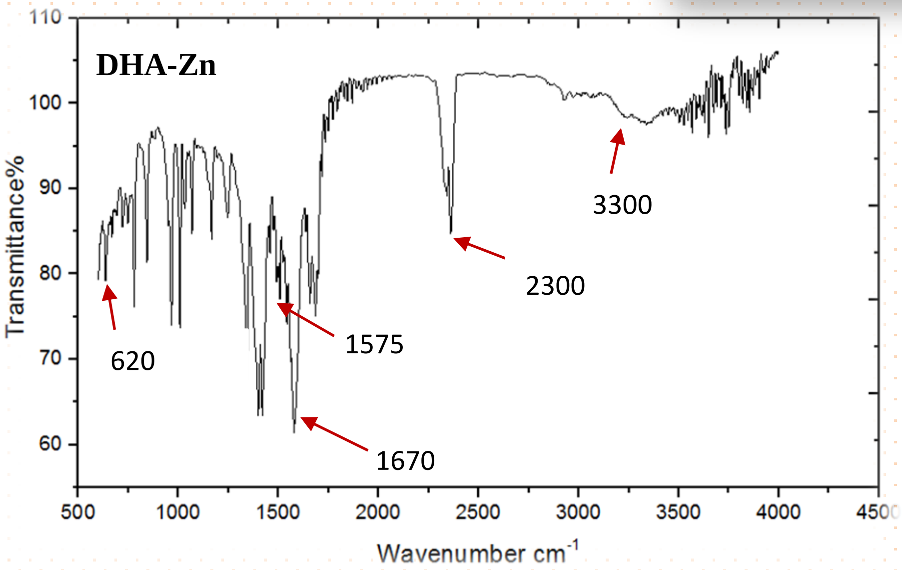
Solid state → **Liquid state**

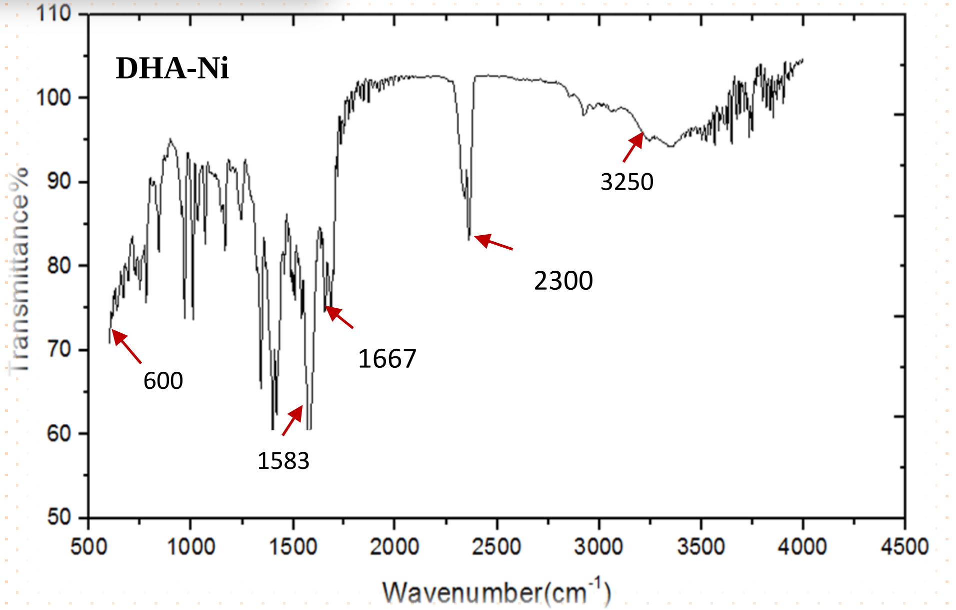
Chelates	**MP**
$Co(DHA)_2.2H_2O$	260
$Ni (DHA)_2.2H_2O$	243
$Zn(DHA)_2.2H_2O$	168
$Mn(DHA)_2.2H_2O$	> 260

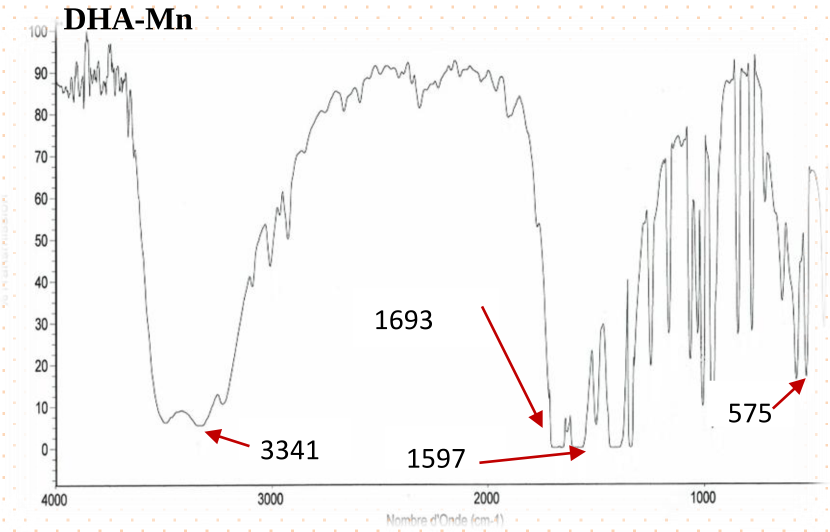
MP → (**Involving**) **Purety**

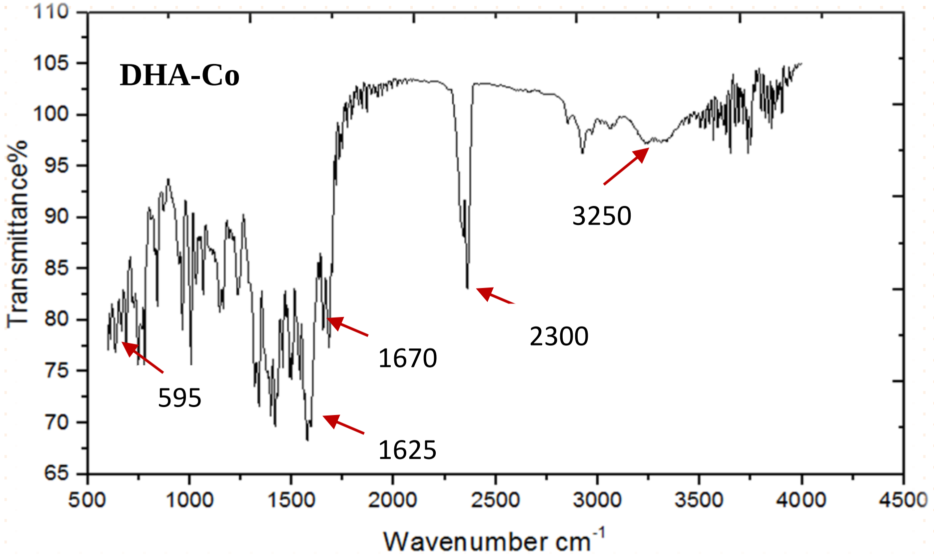


IR spectroscopy



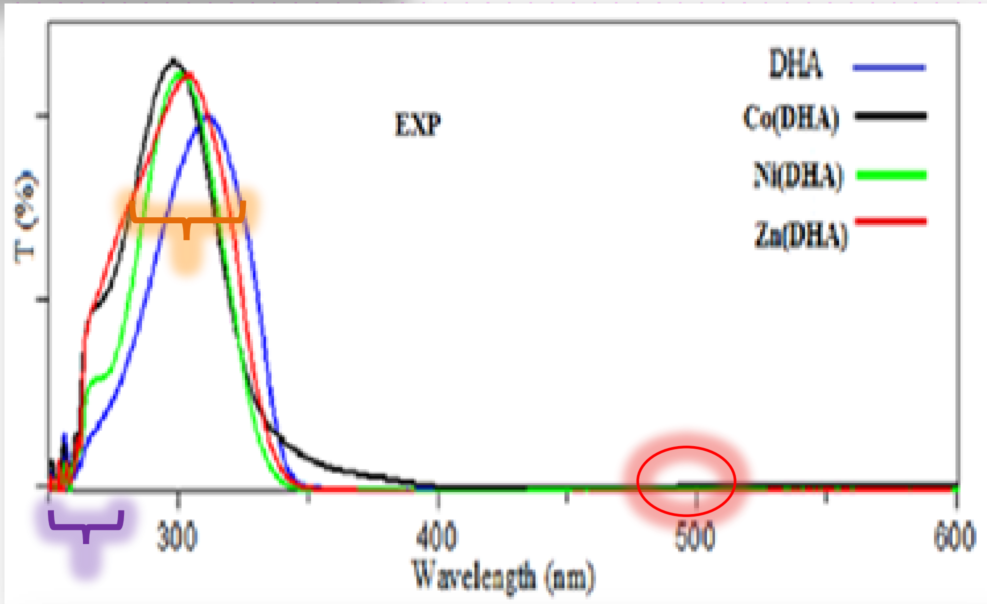




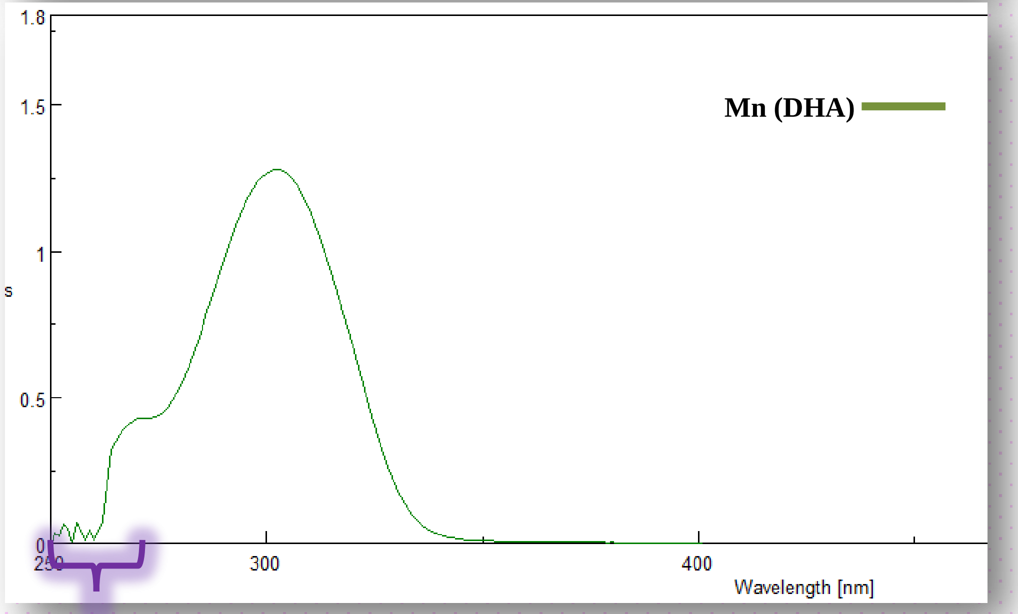


UV-Vis spectroscopy

Commun band in the range of 251-267 nm → C=O



Bathochrom effect → In the range of 285-313 nm



λ = 480 nm → Transition d-d → M–O

In vitro Biological screening

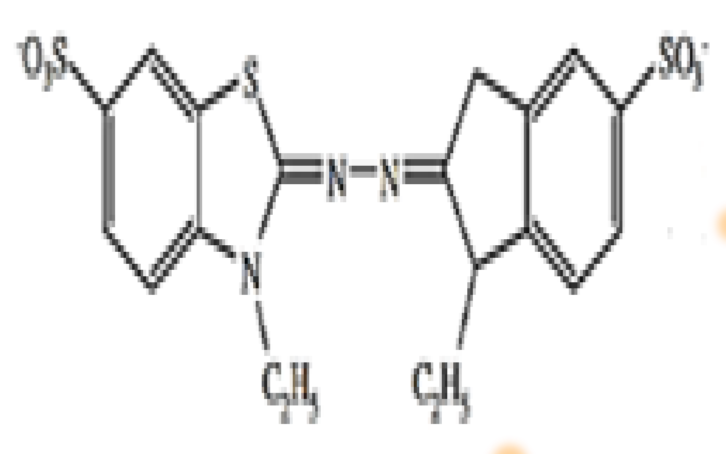
Antioxidant activity

- Scavenging test of ABTS radical
- Fe chelating capacityActivité de chélation des ions ferreux
- Copper chelating ability
- Fe^{2+} chalating ability by UV-VIS
- β- carotène blunching activity
- Antioxydante capacity by copper reducing
- Scavenging test of hydroxyle radical
- Scavenging test of l'hydrogène peroxyde
- Scavenging test of superoxyde (pyrogallol) radical
- Metal chelating actrivity (phénontroline)
- Superoxyde DMSO alcalin test
- Scavenging test of DPPH radical
- Scavenging test of galvinoxyle radical
- Antioxydante capacity by fe reducing

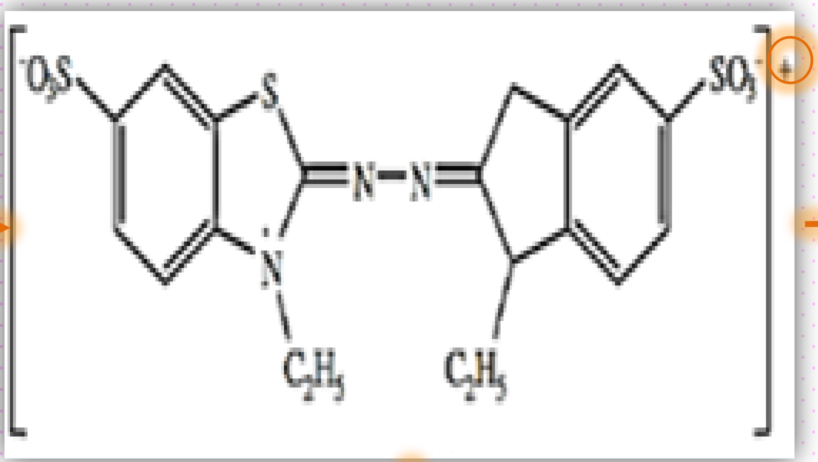
Enzymatic activity

- Uréase
- Acetylcholine estérase
- Alpha amylase
- Butyrylcholine estérase

Scavenging test of ABTS radical

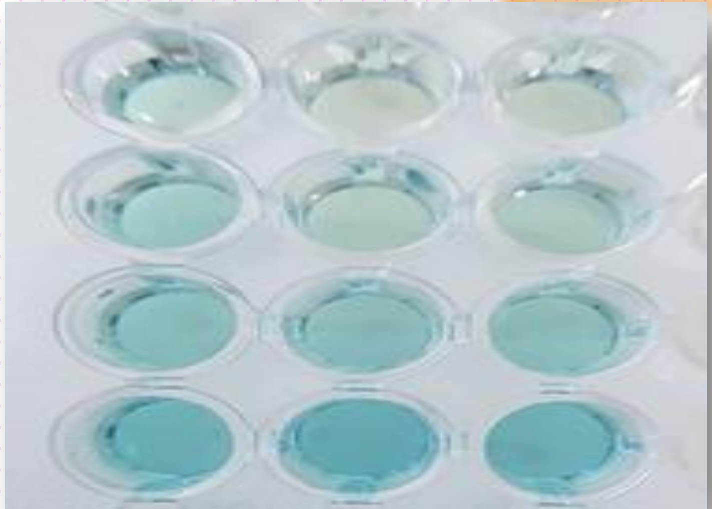


$K_2C_2O_8$

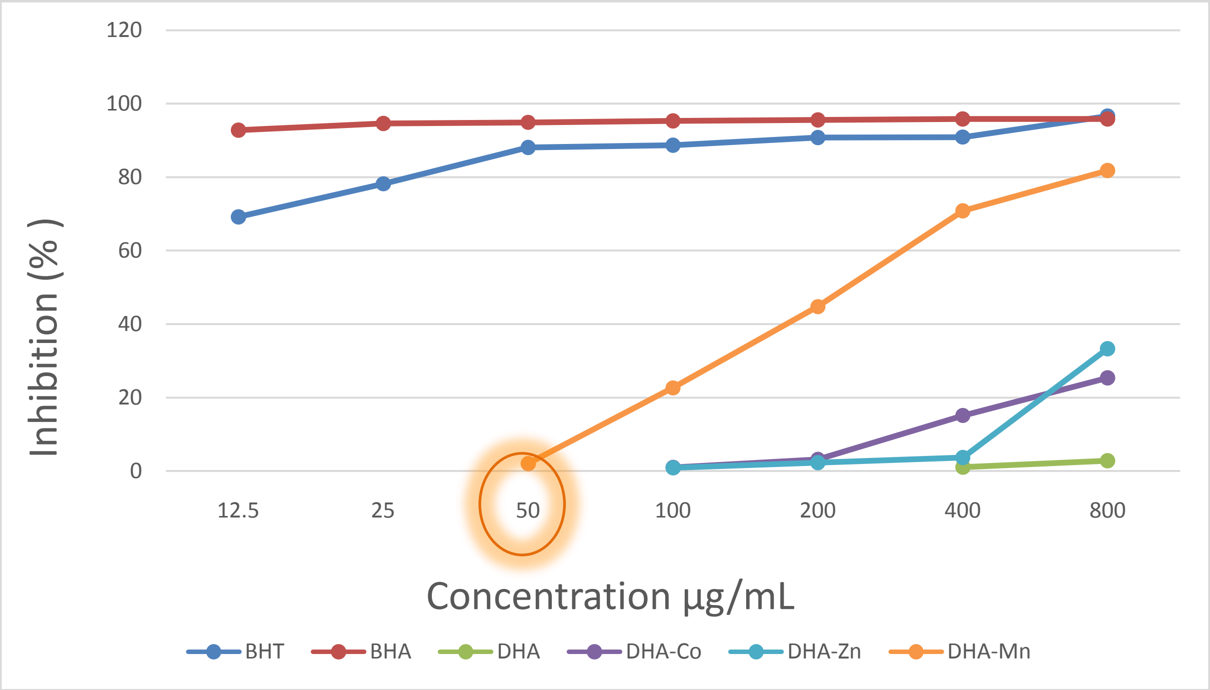


Back to non radical form

Antioxidant agent



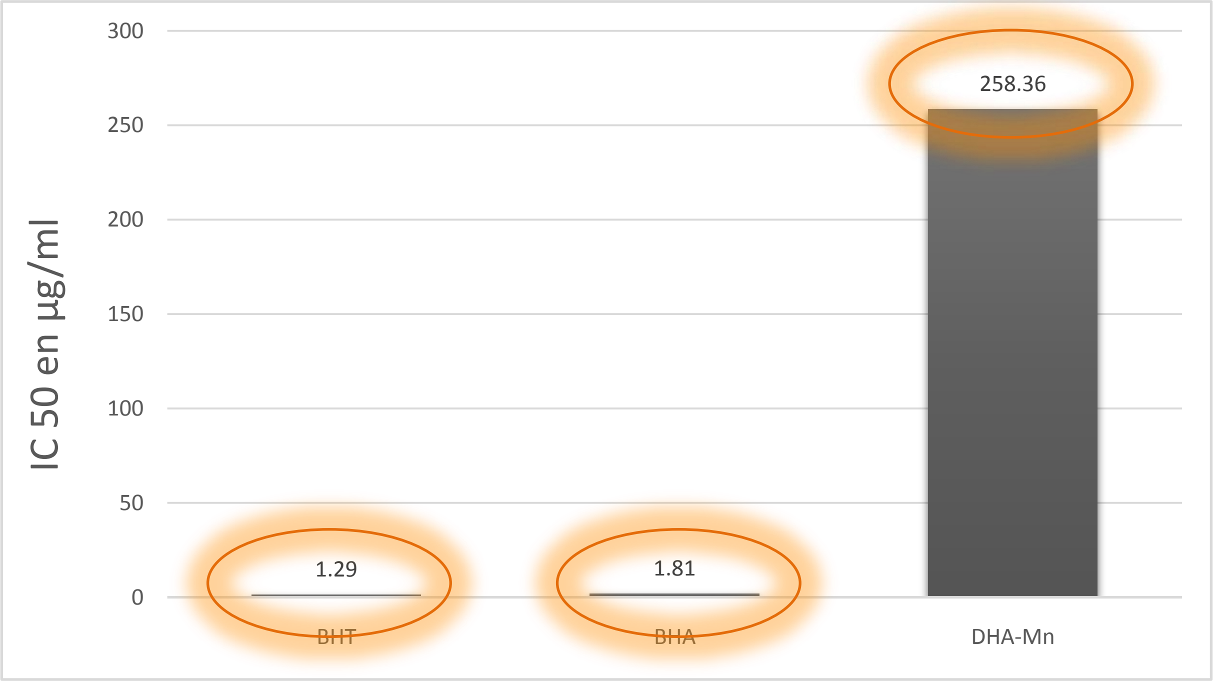
λ= 734 nm



DHA-Mn → **Best réactivity**

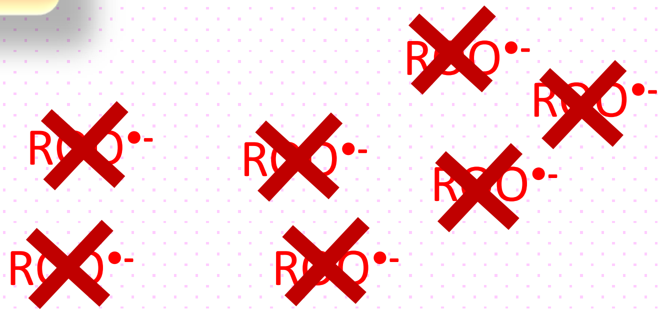
Activity strats at → **50 µg**

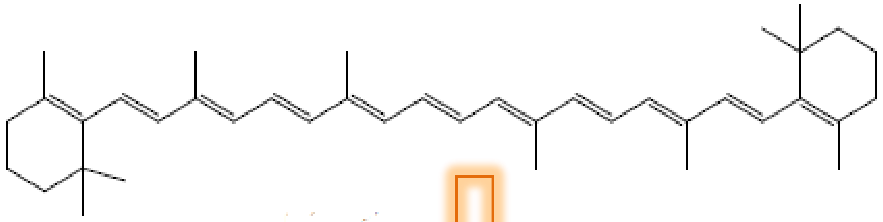
CI50 → **258,36 µg/mL**

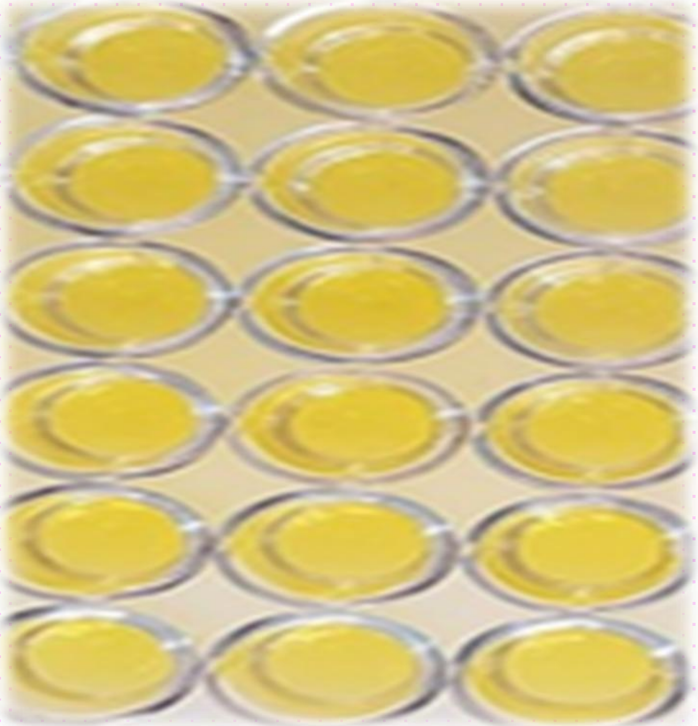


β-carotène blunching test

Oxydation

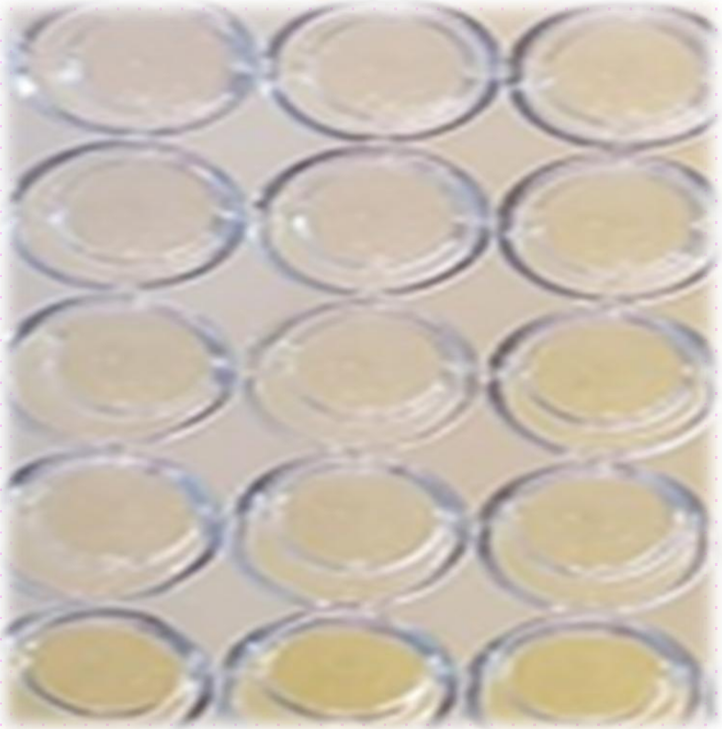




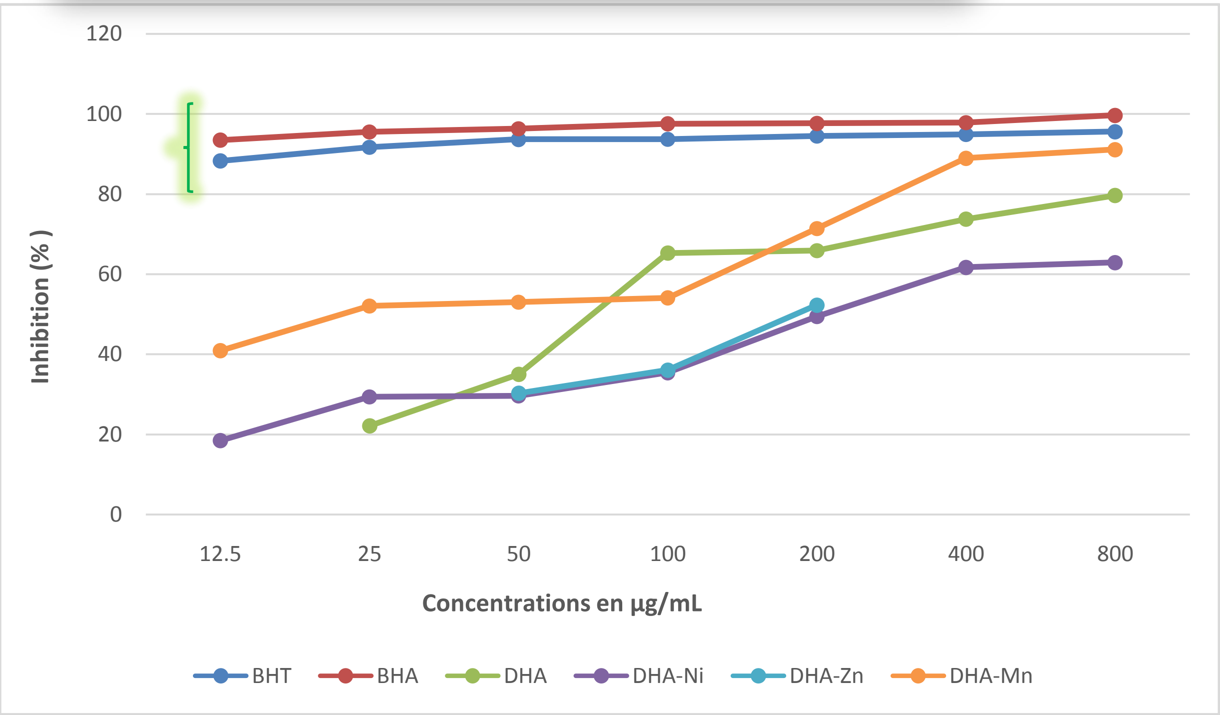


In presence of an antioxidant

λ = 470 nm

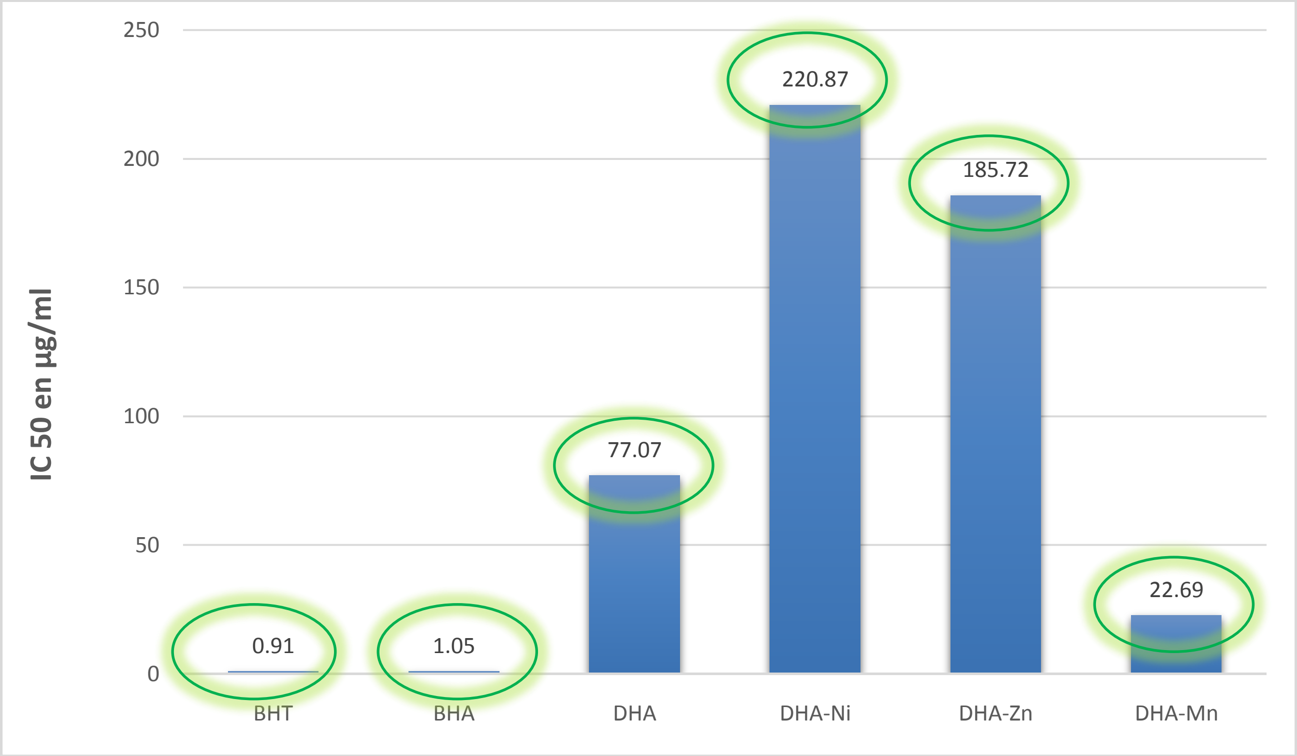


β-carotène blunching test



Chelates + DHA cinetic ⇒ **Slow**

DHA-Mn chelate ⇒ **Better activity**

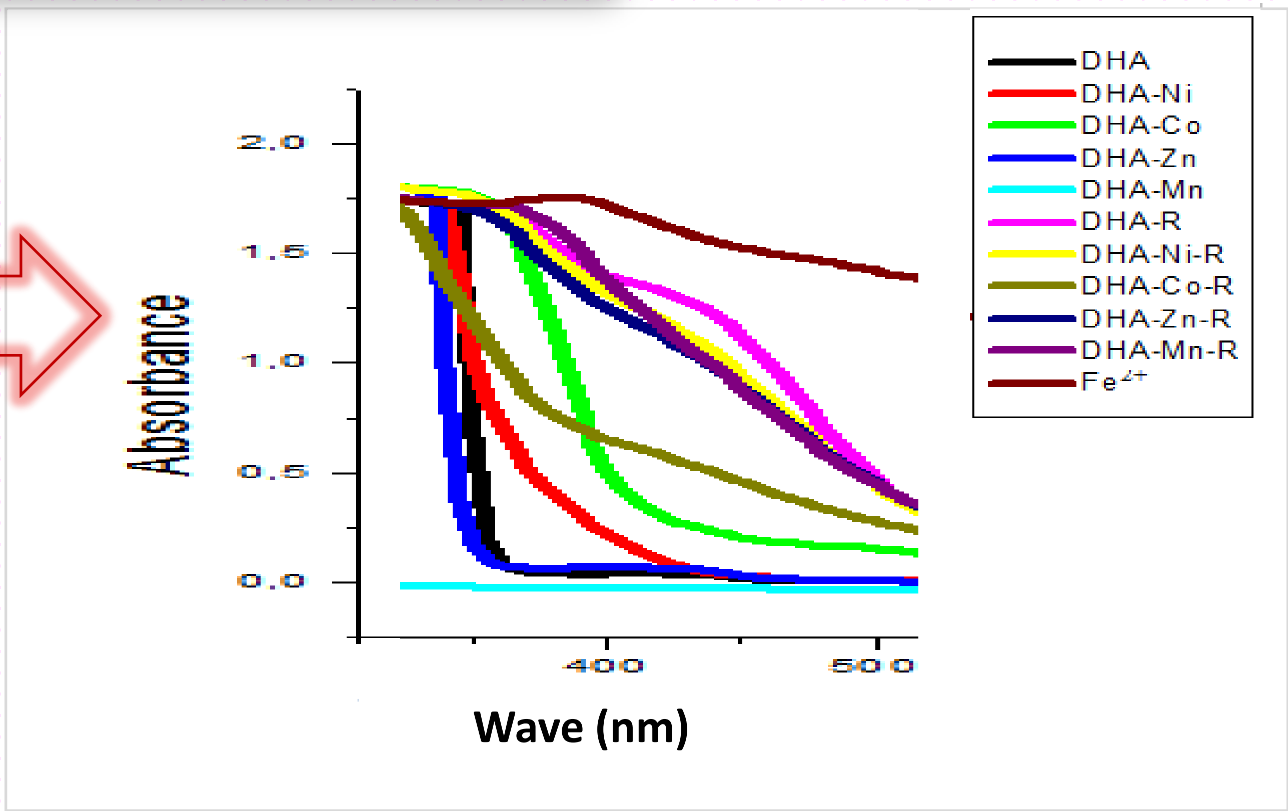


CI50 ⇒ **> BHT and BHA**

Metal chelating activity

Fe^{2+} (Cu^{2+}) + H_2O_2 ⇒ Fe3+ (Cu3+) + OH• + OH

Fe chelating ability by UV-Vis



Fe^{2+} ⇒ Absorbance at 420 nm

Chelates binding to Fe ⇒ Abs ± intense and > to free chalates

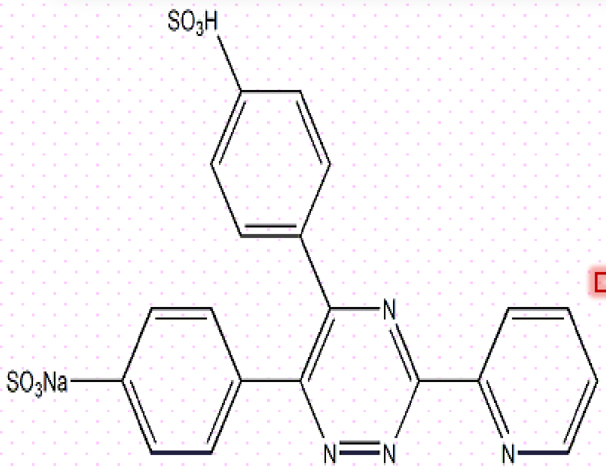
Co-Fe2+ ⇒ Weakest absorbance

⇒ DHA and chelates have a potent fe chelating ability

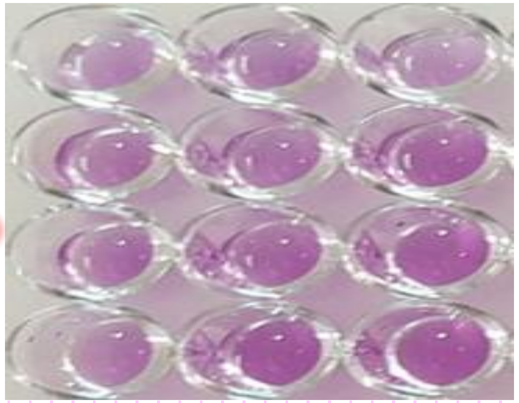
Metal chelating activity

$$Fe^{2+} (Cu^{2+}) + H_2O_2 \Rightarrow Fe3+ (Cu3+) + OH\bullet + OH$$

Fe chelating test

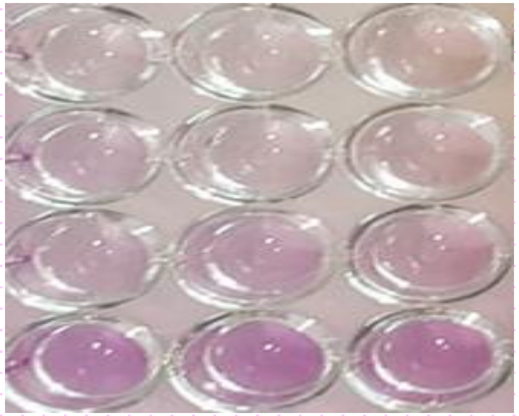


+ Fe^{2+} ⇒



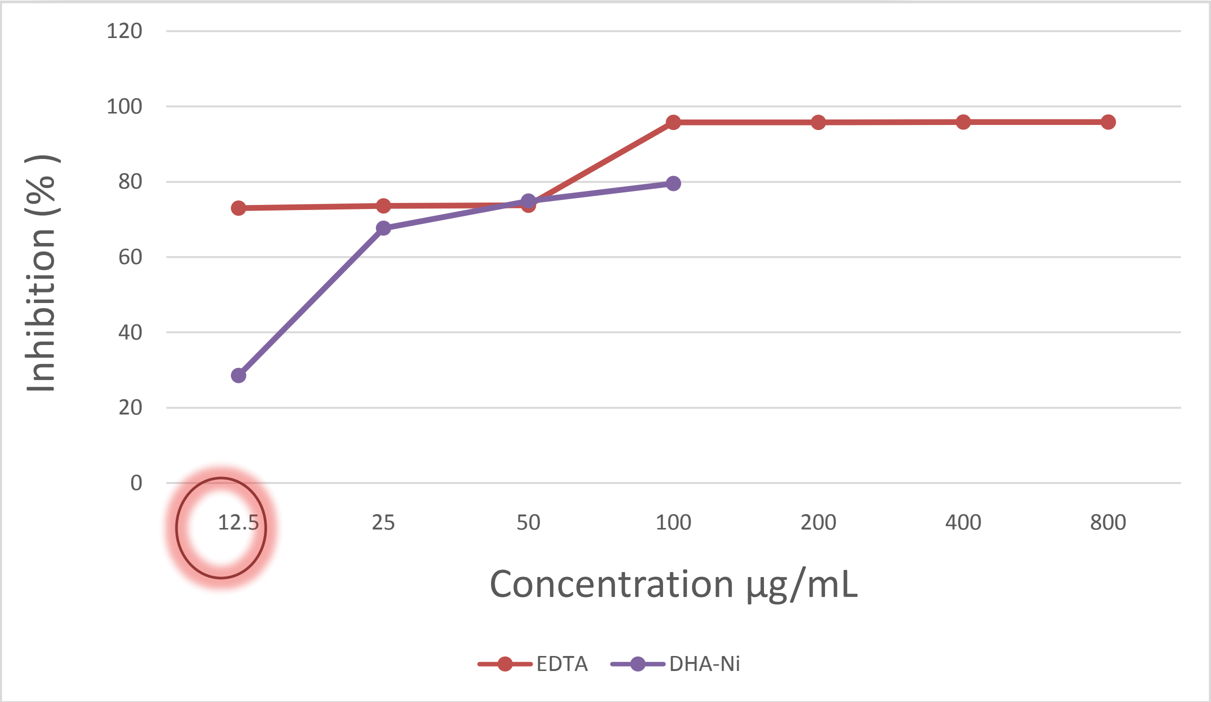
Ferrozine-Fe^{2+}

Chelating agent ⇒



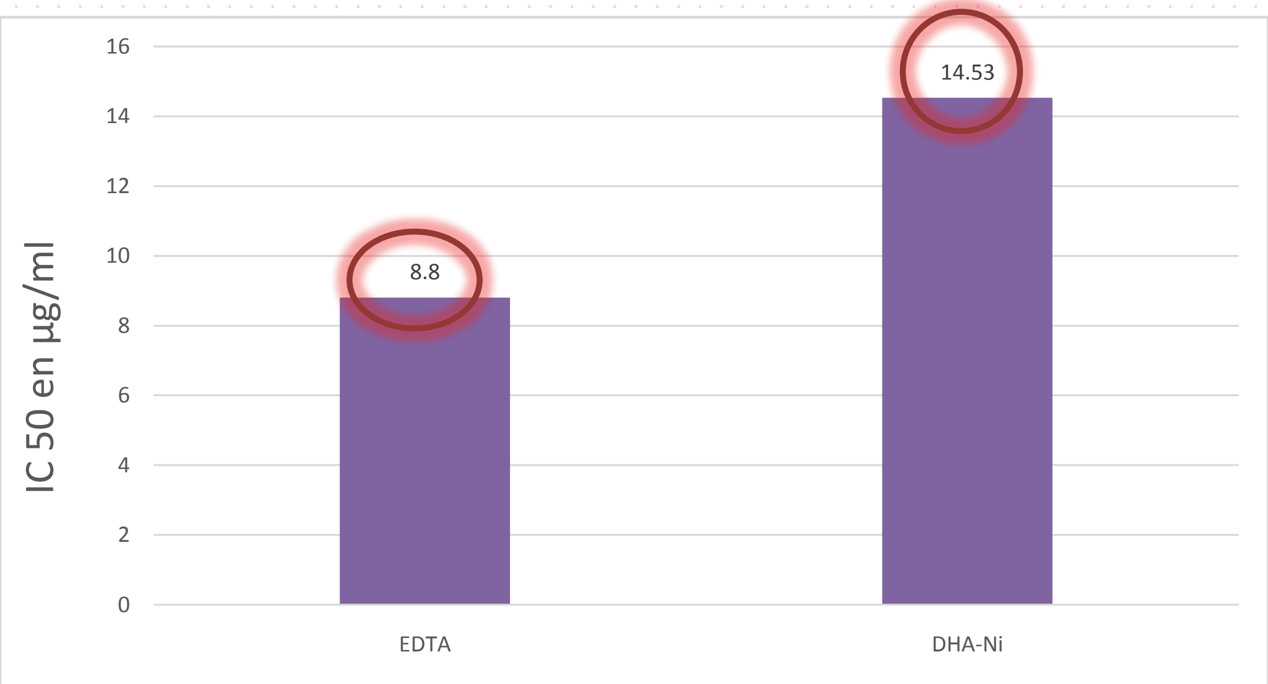
Cloudy chelates

Fe chelating activity



DHA-Ni → **Best activity**

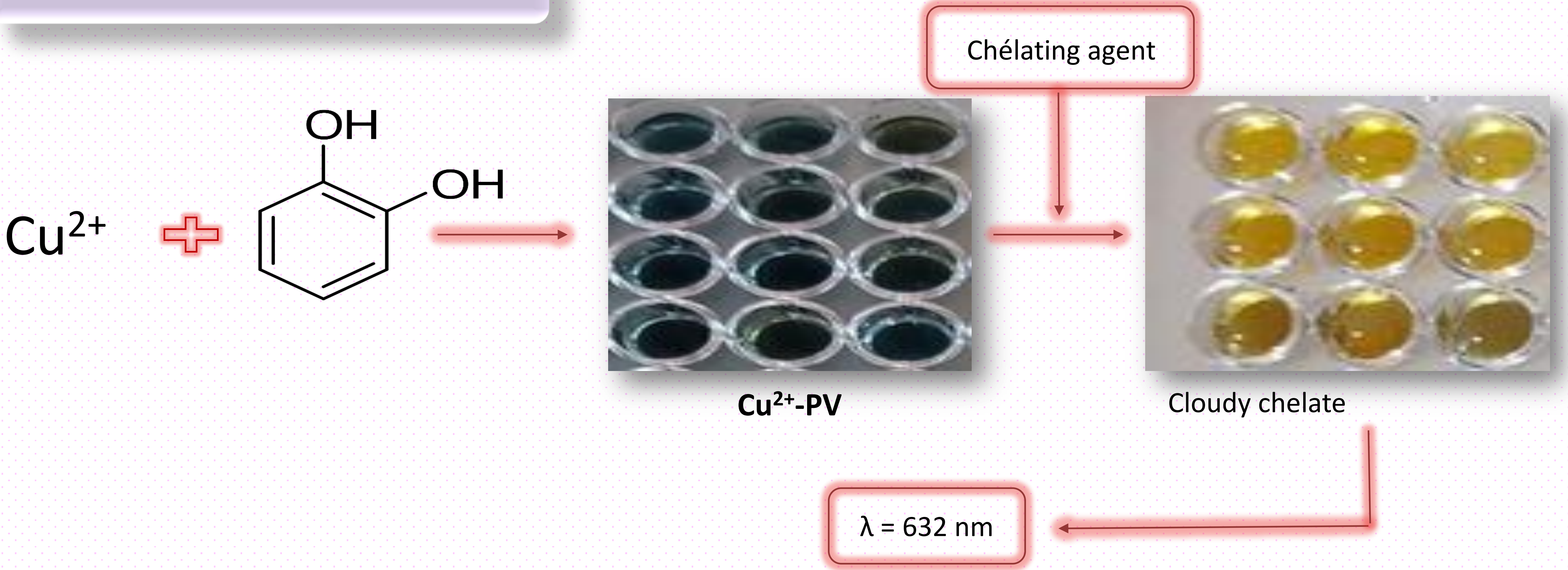
CI50 → **14,53 µg/mL**



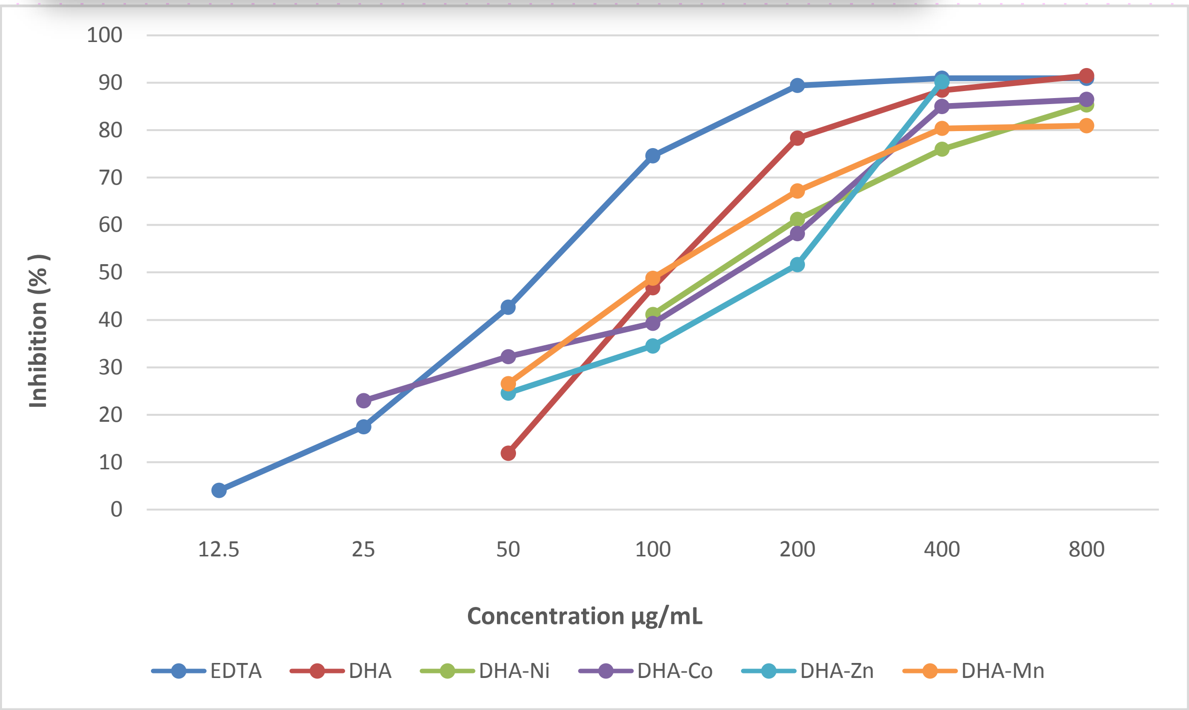
Metal chelating activity

$Fe^{2+} (Cu^{2+}) + H_2O_2$ ⟹ **Fe3+ (Cu3+) + OH• + OH**

Copper chelating activity

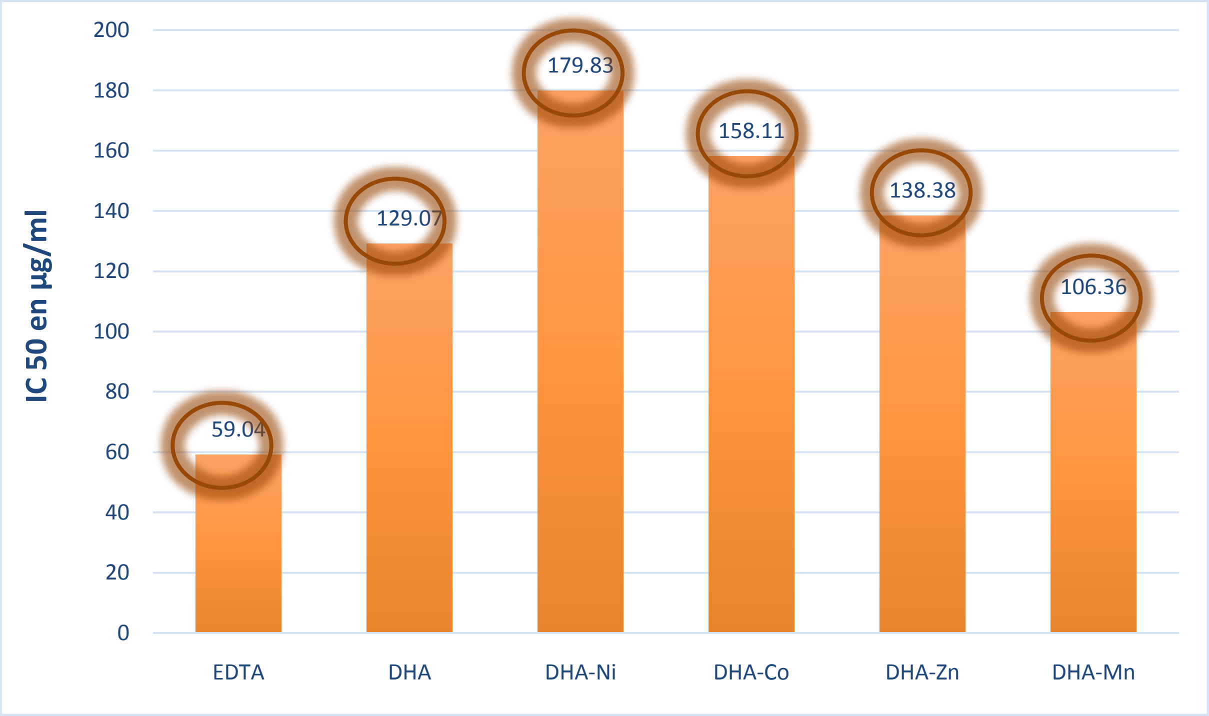


Metal chelating activity



DHA and chelates → Copper chelating agents

DHA-Mn → Best activity



CI50 → 106,36 µg/mL

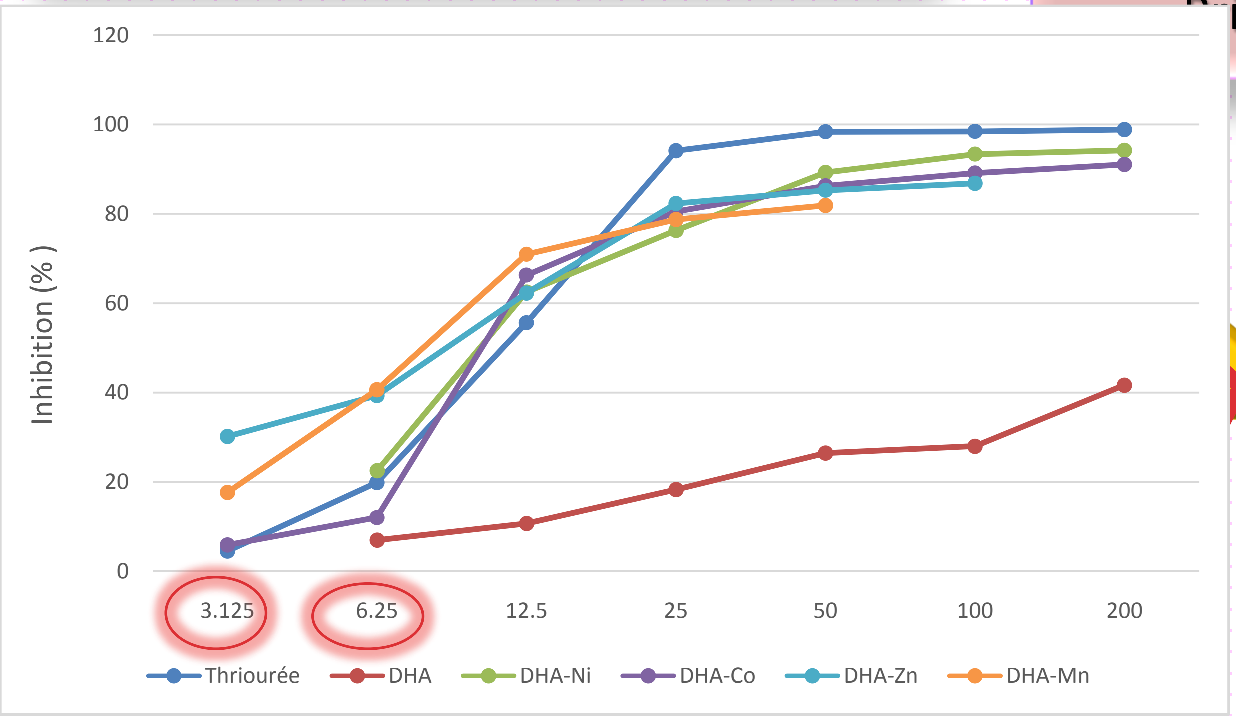
Uréase inhibiting activity

Principe

DHA-Ni, DHA-Co, DHA-Zn et DHA-Mn ⟹ Activity ↗

6,25 µg

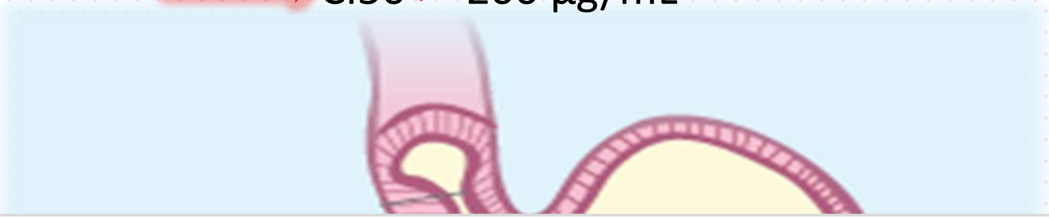
3,125 µg



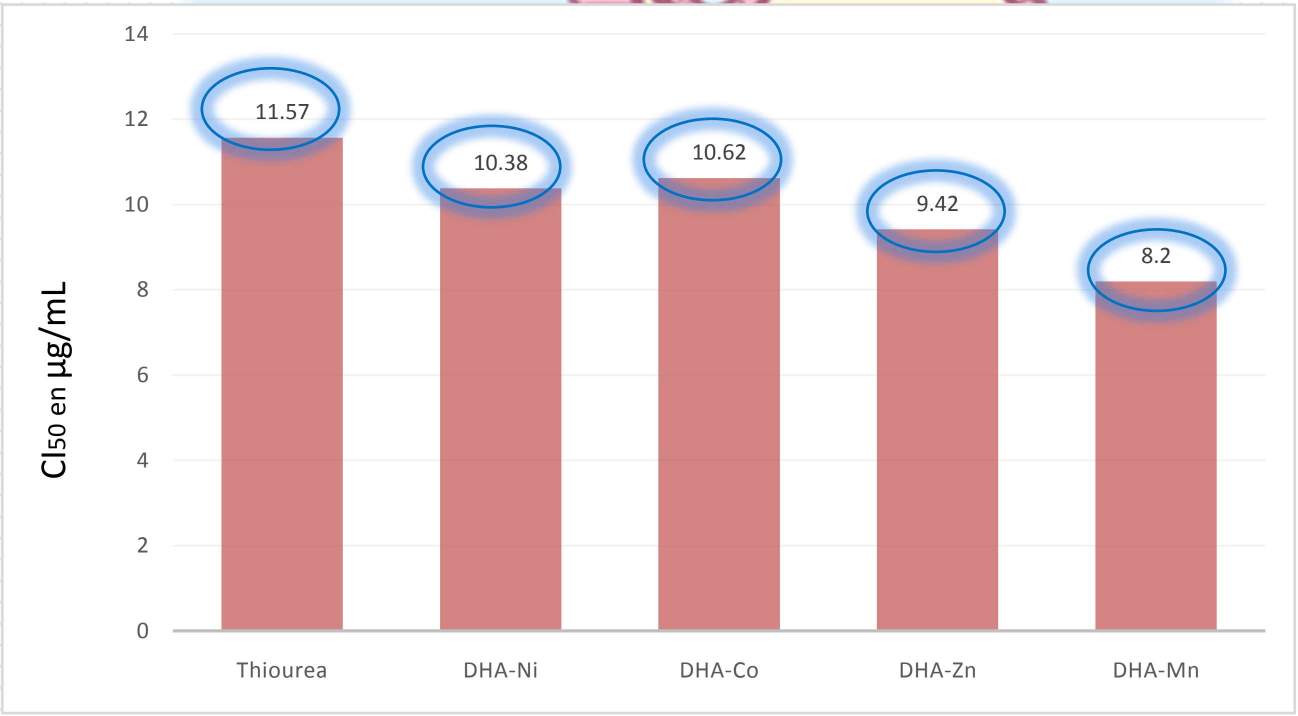
Free DHA

⟹ Activity < Chelates and thioura

⟹ CI50 > 200 µg/mL

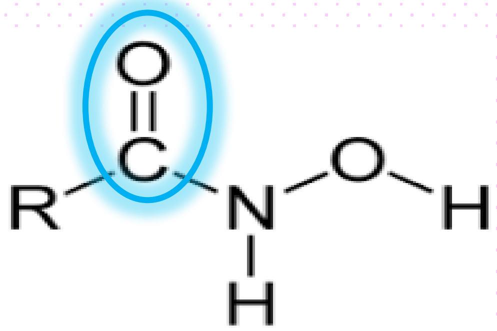


uréase



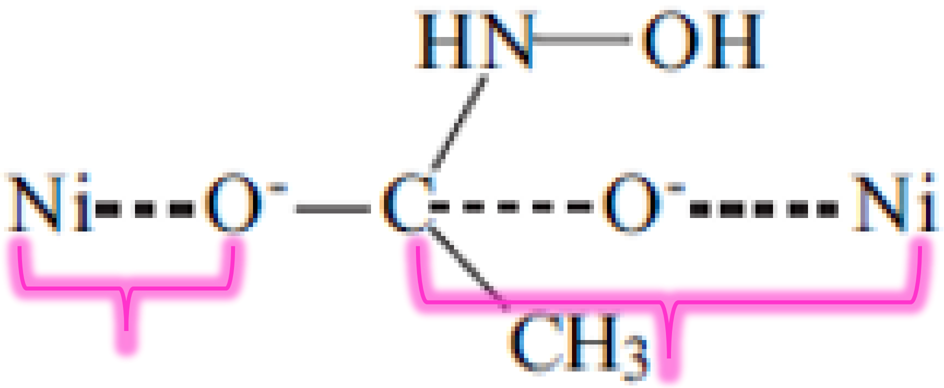
Uréase inhibiting activity

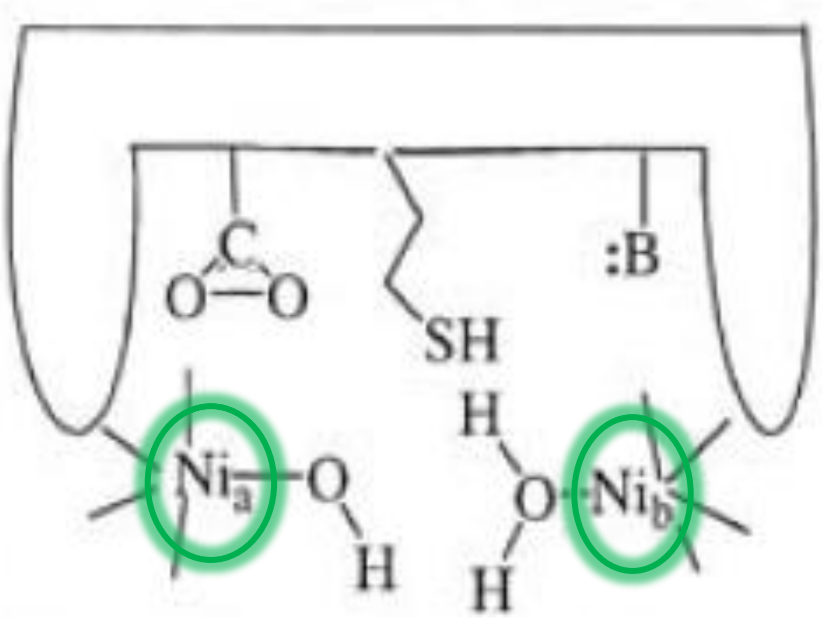
Amtul. Z et al, (2002)



Uréase synthetic inhibitors

Zerner and al model





Ni atoms inactivation

Enzyme activityinhibition

Conclusion

Dehydroacetic acid and its transition metal complexes **(1-4)** were efficiently synthesized, characterized and fully screened for over than 20 *in vitro* biological activities, which exhibit a high urease inhibiting capacity for all chelates, Mn chelate as a hit for antioxidant activity and DHA free ligand as better antimicrobial agent. Discussion on molecular structures and comparison with observed effect helped to explain the structure activity relationship that may or not improve observed therapeutically effect of Dehydroacetic acid by chelating in comparison with DHA free ligand, and suppose that tested compounds adopt different mechanism of action depending on biological application.

In regards of these promising results, kinetic studies, pharmacomodulation of tested oraganometallic complexes to increase medicinal effect and *in vivo* preclinical tests, are recommended as future investigations.

Acknowledgments

Authors would like to thank Algerian Ministry of Higher Education and Scientific Research DGEFS, and the Algerian Directorate General for Scientific Research and Technological Development DGRSDT for financial fund.