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Theoretical investigations of the antioxidant, optical and electronic properties of polyphenols

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Avant-propos

Ce travail de thèse a été réalisé au Laboratoire de Biophysique (l'équipe EA4021) de la Faculté de Pharmacie de l'Université Limoges (France).

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À La mémoire de mon père ANOUAR M'HAMED et de ma grand-mère,

À ma mère El AZZOUZI SAADIA,

À mes frères et sœurs, à mon oncle El ISSATI Mohammed

À toute ma famille,

Abstract

The present PhD deals with the antioxidant activity and the capacity to absorb UV/visible light of polyphenols. Density functional theory (DFT) and time-dependent DFT (TD-DFT), respectively give an accurate description of the experimental data.

In the first section, the structure-antioxidant activity of different series of polyphenols have been established, highlighting the importance of thermodynamic descriptors including bond dissociation enthalpies (BDE), π-electron conjugation, H-bonds, double BDE (BDE_D) and number of OH groups.

In the second section, the excited states and the allowed electronic transitions are accurately evaluated. The number and position of the OH groups, the number of double bonds and the solvent influence the energy of the transitions. Thus, TD-DFT allows for an understanding of the structural parameters which are responsible for the variation of colours in fruits and flowers.

Résumé

La présente thèse discute de l'activité antioxydante et de l'absorption de la lumière UV/visible des polyphénols. La théorie de la fonctionnelle de la densité (DFT pour Density Functional theory) et la DFT dépendante du temps (TD-DFT pour Time Dependant-DFT) donnent une description précise des données expérimentales.

Dans un premier temps, la relation-structure activité (RSA) antioxydante a été établie pour différentes séries de polyphénols. La RSA quantitative souligne l'importance des descripteurs thermodynamiques incluant les enthalpies de dissociation de liaisons (BDE pour Bond Dissociation Enthalpies), la conjugaison des électrons π, les liaisons hydrogènes, les BDE double (BDE_D) et le nombre de groupements OH.

Ensuite, les états excités et les transitions électroniques permises sont précisément estimés. Le nombre et la position de groupements OH, le nombre de doubles liaisons et le solvant ont des effets majeurs sur les énergies de transitions. La TD-DFT permet en partie de mettre en valeur les paramètres physico-chimiques responsables de la variation de couleurs dans les fruits et les fleurs.

Contents

List of Acronyms

HIV	Human Immunodeficiency Virus
UV	Ultraviolet
DFT	Density Functional Theory
TD-DFT	Time-dependent Density Functional Theory
DPPH	2,2-diphenyl-1-picrylhydrazyl
BDE	Bond dissociation enthalpy
DNA	Deoxyribonucleic Acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
RNS	Reactive nitrogen species
HAT	Hydrogen Atom Transfer
ESR	Electron Spin Resonance
AAPH	2,2'-azobis(2-amidinopropane) dihydrochloride
SOMO	Singly Occupied Molecular Orbital
PC-ET	Proton Coupled-Electron Transfer
ET-PT	Electron transfer-Proton transfer
SPLET	Sequential Proton Loss Electron Transfer
NMR	Nuclear magnetic resonance
SCF	Self-consistent-field
RHF	Restricted Hartree-Fock
ROHF	Restricted open HF
UHF	Unrestricted Hartree-Fock
LCAO-MO	Linear Combination of Atomic Orbitals- Molecular Orbital
STO	Slater Type Orbital
CI	Configuration Interaction
MRCI	Multi-reference Configuration Interaction
MRDCI	Multi-reference single and double excitations Configuration-interaction
MP	Møller-Plesset
CASSCF	Complete-Active-Space Self-Consistent-Field
KS	Kohn-Sham

XC	Exchange-correlation
DFT-D	Density Functional Theory for dispersion
LDA	Local Density Approximation
LSDA	Local Spin Density Approximation
GGA	Generalized Gradient Approximation
GEA	Gradient expansion approximation
SIC	Self-interaction Correction
GS	Ground state
ES	Excited state
PCM	Polarizable Continuum Mode
IEF-PCM	Integral equation formalism- Polarizable Continuum Mode
AA	Arachidonic Acid
QM/MM	Quantum mechanics/Molecular mechanics
EPR	Electron Paramagnetic Resonance
ZPE	Zero point energy
TS	Transition state
IRC	Intrinsic Reaction Coordinate
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
QSAR	Quantitative structure-activity relationship
AF	Adduct formation
MLR	Multiple Linear regression
RSS	Residual sum of squares
MSS	Model sum of squares
SEP	Standard error of prediction
SE-CI	Single excitation-Configuration interaction
SPR	Structure property relationship

Introduction

Polyphenols are organic compounds possessing at least one phenol moiety in their chemical structures. Numerous different structures exist; they are subdivided into various classes and subclasses including flavonoids, phenolic acids, lignans, procyanidins and tannins. They are largely distributed in fruit (e.g., apples, kiwis, tomatoes, berries and grapes), vegetables (e.g., broccoli, artichokes and pepper), spices (e.g., curcumin and nutmeg), beverages (e.g., tea, fruit juices, wines and chocolate) and different oils (e.g., olive, and argan). They are known for their biological activities, (e.g, anti-HIV, anti-atherosclerosis, anti-cancer and anti-inflammatory). They are powerful antioxidants according to their capacity to i) directly scavenge free radicals, ii) inhibit lipid peroxidation, iii) chelate metal and iv) inhibit enzymes involved in oxidative stress. In addition to their biological activities, the colour of flowers, fruit and vegetables can be partly explained by the capacity of these compounds to selectively absorb UV/visible light.

Over the past years a large number of joint experimental and theoretical investigations have focused on the antioxidant behaviour of polyphenols. However, a systematic theoretical study on the structure activity relationship of different series of polyphenols, for their capacity to scavenge free radicals was not carried out. In a second step we will establish structure property relationship of polyphenols, for their capacity to absorb UV/visible light. This rationalizes the variation of colours, which are found for different plants. The methodology is generally based on the (time-dependent) density functional theory, (TD-)DFT formalism; alternative (semi-empirical) approaches are explored for comparison. The experimental results that are presented in this work were obtained by other researchers of the EA4021 lab, in the pharmaceutical college of the University of Limoges.

Chapter 1 is a bibliographic section, which details i) oxidative stress (free radicals and the systems of defense), ii) polyphenols (classes and subclasses, distribution in the plant

kingdom, beneficial effects, biological activities and structure antioxidant-activity relationship) and iii) colour variation (e.g., in fruit and flowers) attributed to polyphenols and their UV/visible spectra *vs* molecular structures.

Chapter 2 gives an overview of the basic concepts of quantum chemistry. This chapter is divided in three main sections. In the first one, the Schrödinger equation, the Born-Oppenheimer approximation and the Hartree-Fock method are briefly described. The basic notions of DFT are treated in section 2. The last section focuses on electronic spectroscopy, excited states and time-dependent DFT (TD-DFT).

Chapter 3 presents the theoretical results related to the structure-activity relationship of polyphenolic compounds. The results are presented as they are published or submitted in international scientific journals. We first present a study concerning the stability of free radicals. This has been published in International Journal of Quantum Chemistry (IJQC) and I mainly participated in the evaluation of the chemical behaviour of DPPH (2,2-diphenyl-1-picrylhydrazyl). This stable free radical is not naturally produced in the organism but it is largely used to evaluate *in vitro* the antioxidant properties of new compounds. We then present a joint theoretical and experimental study (published in the Journal of Physical Chemistry A) on the antioxidant activity of a new series of nine $(guaiacol)_{n=1-4}$ oligomers. The goal is to understand the electronic structure-activity relationship and the role of the guaiacyl moiety. Bond dissociation enthalpies (BDE), double BDE and number of OH groups of these oligomers are correlated to their capacity to scavenge DPPH. Finally a rationalization of the structure activity relationship is established for a large series of polyphenols (more than 40 different compounds). In this article, which is in preparation, different (major and minor) descriptors are described. This helps to rationalize the free radical scavenging capacity and to predict the antioxidant capacity of new compounds.

Chapter 4 deals with the UV/visible spectra of polyphenols and their role in colour variation of flowers, fruit and vegetables. The absorption of light induces electronic transitions from the ground state to various excited states, where the energy and strength of the transitions depend sensitively on the chemical structure. We will show that TD-DFT calculations allow for an accurate description of the optical properties of polyphenols. We investigate a large series of polyphenols and on a small series of $(guaiacol)_{n=1-4}$. The role of substitution, solvent, chain length and π-electron conjugation is discussed.

Chapter 1: Oxidative stress, Antioxidants, Polyhenols and Colours

Free radicals and oxidative stress

I. Free radicals

In molecular systems, the cohesion between atoms is attributed to different repulsive and attractive interactions. The nature of the bond depends on the atoms and their physico-chemical properties (e.g., electronegativity). The excitation of molecules by radiations (radiolysis, sun light, ultraviolet…) or by collision (e.g., thermal agitation, electronic impact…) leads to bond cleavage in which each bonded atom may keep their own electron (homolytic dissociation), to form free radicals. Free radicals are atoms or molecules possessing an unpaired electron in their outer shell (valence electrons), usually the radicals are quoted $R^{\bullet}$. The presence of the unpaired electron in the outer shell makes the free radicals highly reactive. Free radicals attack the neighbouring molecules, which is particularly damageable when free radicals react with biological molecules as DNA, proteins and lipids.

When considering a living organism, free radicals may be endogenous or exogenous. The endogenous free radicals are mainly originated by the respiratory chain, the activated leukocytes and enzymes. The exogenous free radicals might originate from cigarette smoke, pollution, radiation (in particular UV light) and stress. They are generally toxic and are the key agents of the oxidative stress, which is defined as an overproduction of free radicals.

Most of the free radicals present in the organism are oxygen-centred and are so-called reactive oxygen species (ROS). The most common reactive oxygen species are the superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), peroxide radicals ($ROO^{\bullet}$) and alkoxy radicals ($RO^{\bullet}$).

I.1. Superoxide radical

During the respiratory chain process, the triplet oxygen $^3O_2$ is reduced by cytochrome:

$$O_2 + 4e^- + 4H^+ \xrightarrow{\text{Cytochrome oxydase}} 2H_2O \quad \text{(reaction I. 1)}$$

If one of the electrons escape from the reaction system above (I. 1), the superoxide anion $O_2^{-\bullet}$ is formed:

$$O_2 + e^- \xrightarrow{\text{Cytochrome oxydase}} O_2^{-\bullet} \qquad \text{(reaction I. 2)}$$

The superoxide formation is catalyzed by enzymatic systems e.g., xanthine/xanthine oxidase. In the presence of the dioxygen, xanthine oxidase catalyzes the oxidation of the xanthine to uric acid.

Due to its very short lifetime, $O_2^{-\bullet}$ is not considered as a direct source of solute degradation. However it is a major intermediate for the production of the other free radicals.

I.2. Hydrogen peroxide

In the presence of protons, the superoxide radical forms hydrogen peroxide H_2O_2. The reaction is catalyzed by the superoxide dismutase (SOD):

$$2O_2^{-\bullet} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \qquad \text{(reaction I. 3)}$$

H_2O_2 is not a free radical but is a reactive intermediate in the free radical production.

I.3. Hydroxyl radical

The reaction between $O_2^{-\bullet}$ and H_2O_2 (Haber-Weiss reaction) forms the very toxic free hydroxyl radical ($^{\bullet}OH$):

$$O_2^{-\bullet} + H_2O_2 + H^+ \rightarrow O_2 + HO^{\bullet} + H_2O \qquad \text{(reaction I. 4)}$$

The hydroxyl radical can also be generated by the degradation of hydrogen peroxide in the presence of ferrous iron (Fenton reaction):

$$H_2O_2 + Fe^{2+} \rightarrow HO\cdot + HO^- + Fe^{3+} \qquad \text{(reaction I. 5)}$$

$^{\bullet}$OH radicals may also be produced by the radiolysis of water (induced by X and γ-rays).

Among all the ROS that are produced in the organism, $^{\bullet}$OH is the most reactive and the most toxic. It is known to originate degradation of DNA, lipid membranes and proteins [1].

I.4. Peroxy radicals

Peroxy radicals are formed by the addition of molecular oxygen to the carbon-centred free Radicals:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad \text{(reaction I. 6)}$$

If R is a lipid chain (LH) then the radical is quoted $LOO^{\bullet}$ as those produce during the lipid peroxidation process.

I.5. Alkoxy radicals

Alkoxy radicals ($RO^{\bullet}$) are usually formed by the degradation of organic peroxide ROOH e.g., in the presence of metals:

$$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + HO^{-} + Fe^{3+} \quad \text{(reaction I. 7)}$$

They are highly reactive as $^{\bullet}OH$.

I.6. Other radicals

There also exist the carbon-centered radicals (e.g., $^{\bullet}CH_2OH$ and $^{\bullet}CH_3O$ as produced during intoxication to ethanol and methanol, respectively or $L^{\bullet}$ as produced during lipid peroxidation).

Reactive nitrogen species (RNS) are also produced in the organism. Nitric oxide ($NO^{\bullet}$) are produced by endothelium, macrophages, neutrophils, and brain synaptosomes [2]. The direct toxicity of nitric oxide is low but is greatly enhanced when reacting with $O_2^{-\bullet}$ to form the peroxynitrite anion (ONOO-):

$$O_2^{-\bullet} + NO^{\bullet} \rightarrow ONOO^{-} \quad \text{(reaction I. 8)}$$

Beckman *et al.* demonstrated that $ONOO^{-}$ reacts slowly with most of the biological molecules making selective oxidation [2].

II. Lipid peroxidation

Lipids (LH) are the main components of cell membranes and lipoproteins. Their oxidation leads to the formation of free radicals and instable intermediates, which are responsible for e.g., the decrease of fluidity in membranes.

Lipid peroxidation of polyunsaturated fatty acids proceeds by a free radical chain reaction through three major steps: initiation, propagation and termination.

(i) Initiation:

Initiation is usually originated in the organism by $^{\bullet}OH$ radicals that are produced either by radiation or in the presence of metal ions. $^{\bullet}OH$ radicals attack lipid chain by H-atom abstraction to form the carbon-centred lipid radical ($L^{\bullet}$):

$$LH + HO^{\bullet} \rightarrow HOH + L^{\bullet} \qquad \text{(reaction I. 9)}$$

(ii) Propagation:

Lipid radicals then react with O_2 to form fatty acid peroxyl radical ($LOO^{\bullet}$).

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \qquad \text{(reaction I. 10)}$$

Afterward the radical propagates from chain to chain by H-atom transfer (HAT):

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \qquad \text{(Reaction I. 11)}$$

In the presence of ferrous iron, alkoxy radicals may be produced:

$$LOOH + Fe^{2+} \rightarrow HO^{-} + Fe^{3+} + L0^{\bullet} \qquad \text{(reaction I. 12)}$$

(iii) Termination

The reaction is completed when two radicals react to form non-radical species:

$$\begin{aligned} &L^{\bullet} + L^{\bullet} \rightarrow L - L \\ &LO^{\bullet} + L^{\bullet} \rightarrow LOL \\ &LOO^{\bullet} + LOO^{\bullet} \rightarrow LOOL + O_2 \qquad \text{(reaction I. 13)} \\ &LOO^{\bullet} + L^{\bullet} \rightarrow LOOL \end{aligned}$$

III. Defense systems against free radicals

To scavenge free radicals and to decrease the effect of oxidative stress three natural defense systems exist: antioxidant enzymes, metal-chelating proteins and natural antioxidants.

III.1. Antioxidant Enzymes

Three enzymes are mainly responsible for the inhibition of oxidative stress in the organism. The superoxide dismutase (SOD) increases the dismutation rate of superoxide radical anion (reaction I. 14) by a factor of 10^4-10^5 compared to a noncatalyzed dismutation:

$$2O_2^{-\cdot} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad \text{(reaction 1.14)}$$

SOD dismutes two superoxide molecules per reaction loop. However, the dismutation of radical anion superoxide $O_2^{-\bullet}$ leads to the formation of H_2O_2, which also contributes to cell toxicity [3].

Catalase catalyses the transformation of H_2O_2 into two water molecules and O_2:

$$2H_2O_2 \xrightarrow{Catalse} O_2 + 2H_2O \qquad \text{(reaction I. 15)}$$

Nevertheless, its role is limited, since the enzyme is confined almost exclusively into red blood cells and peroxisomes. It is absent or in small concentration in extracellular fluid [3-5]. It seems that the suppression of H_2O_2 and ROOH as well, is mainly due to the seleneoenzyme glutathione peroxidase (Se-GPx). This enzyme consists in four identical subunits, each one containing a Se-atom. It catalyzes the reduction of H_2O_2 as well as ROOH.

These three enzymes play a major role in the protection of sites that are particularly exposed to oxidative stress.

III.2. Metal-Complexing Proteins

In extracellular fluids, there are no such enzymes and GSH concentration are very low. Nevertheless, extracellular fluids frequently receive superoxide anions as well as hydrogen peroxide fluxes stemming from activated phagocytes. Gutteridge showed the importance of complexation with metal-chelating proteins [6].

III.3. Vitamin Antioxidants

When large antioxidant molecular systems (enzymes and metalloproteins) are not able to regulate the oxidative balance and decrease the oxidative stress, smaller molecules may act as free radical scavengers. Most of these antioxidants are known as vitamins.

Vitamin E or α-tocopherol (Fig. I.1) is a fat-soluble vitamin present in cell membranes. It is a powerful antioxidant, mainly a lipid peroxidation inhibition since its traps peroxyl free radicals $ROO^{\bullet}$, thus breaking the chain reaction. It has been demonstrated that when free radicals are formed in a tissue, the level of vitamin E decreases in this tissue [7].

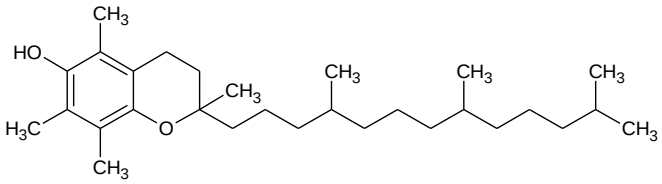

Figure I.1: Molecular structure of α-tocopherol (or vitamin E)

In the nature, eight forms of vitamin E exist: four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ). Tocopherol possesses three asymmetrical carbon atoms, but in the nature only the R, R, R stereoisomer is found. The activity of vitamin E derivatives depends on the number of methyl groups in the aromatic ring, the presence or the absence of double bonds in the side chain and stereoisomery. The most active stereoisomer is (R,R,R)-α-tocopherol.

Ascorbic acid or vitamin C (Fig. I.2), a water-soluble molecule (insoluble in lipid) is present in intra and extracellular fluids but absent in membranes and lipid regions. Vitamin C plays a major role in free radical scavenging, particularly in lipid peroxidation inhibition [8]. It is known to regenerate vitamin E by HAT.

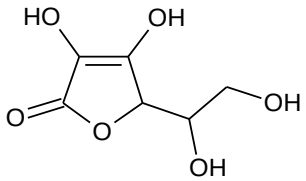

Figure I.2: Molecular structure of ascorbic acid (or vitamin C)

Carotenoid derivatives are also known to decrease oxidative stress. β-carotene (Fig. I.3) is considered as the most active carotenoid vitamins. This vitamin is well known for its provitamin A action. Burton and Ingold suggested that the antioxidant activity of β-carotene acts in biological compartments under conditions of low oxygen partial pressure [9, 10]. β-carotene has a long hydrocarbon chain with eleven conjugated double bonds. It can react with $ROO^{\bullet}$ radicals to form a radical derived from β-carotene, with unpaired electron stabilized by π-conjugation.

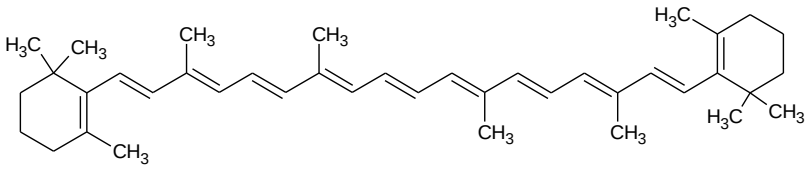

Figure I.3: Molecular structure of β-Carotene

Other kind of vitamins may exhibit antioxidant activities, particularly vitamin B2 (riboflavin) and folic acid. Natural polyphenols e.g., flavonoids are also known as powerful free radical scavengers. Flavonoids were historically considered as vitamin P but they are not classified anymore as vitamins. The next section is dedicated to polyphenols, as a source of antioxidants.

Polyphenols as antioxidants

I. Chemical Structure

Polyphenols constitute one of the most famous groups of phytochemical compounds. They are largely distributed in the plant kingdom, and subsequently in human diet. A polyphenol is a compound with at least one phenolic ring. Depending on their chemical skeleton and substitution they belong to different sub-classes including, phenolic acids, flavonoids, isoflavonoids, lignans and flavonolignans

Actually polyphenols are also defined according to their biosynthesis. They are an important group of second metabolites formed in plants and microorganisms. They are produced from two metabolic pathways: the shikimate and the acetate pathways. The latter (using acetic acid as a precursor) produces phenolic compounds but also terpenoids and fatty acids. It is not as specific as the former pathway. The shikimate pathway starts from the condensation of phosphoenolpyruvate and erythrose-4-phosphate to form a 7-atom-compound which undergoes cyclization to form shikimate (Fig. I.4). This intermediate combines another phosphoenolpyruvate moiety to form chorismic acid and cinnamic acid (Fig. I.4). Cinamic acid may form coumarins, lignans, xanthones, diarylheptanoids and finally flavonoids.

This pathway allows an incredible number of branches allowing a huge amount of different molecular structures. Each step (bond formation, bond cleavage…) is regulated by specific enzymes.

I.1. Small phenolic compounds

There exist only a few natural simple phenols (i.e., compounds with only one aromatic ring). Resorcinol (1,3-dihydroxybenzene) and phloroglucinol (1,3,5-trihydroxybenzene) (Fig. I.4) are formed in the resin and bark of fruit trees, respectively.

Phenolic acids and hyrdoxybenzoic aldehydes constitute a group characterized by the presence of a carboxylic group and an aldehyde group, respectively (e.g., gallic acid, vanillic acid and vanillin as hydroxybenzoic aldehydes) (Fig. I.4).

Cinnamic acids and coumarins have a C6-C3 skeleton. Coumarins possess a hetero-ring (e.g., umbelliferone) (Fig. I.4). The most common cinnamic acids are cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid, 5-hydroxyferulic acid and sinapic acid (Fig. I.4).

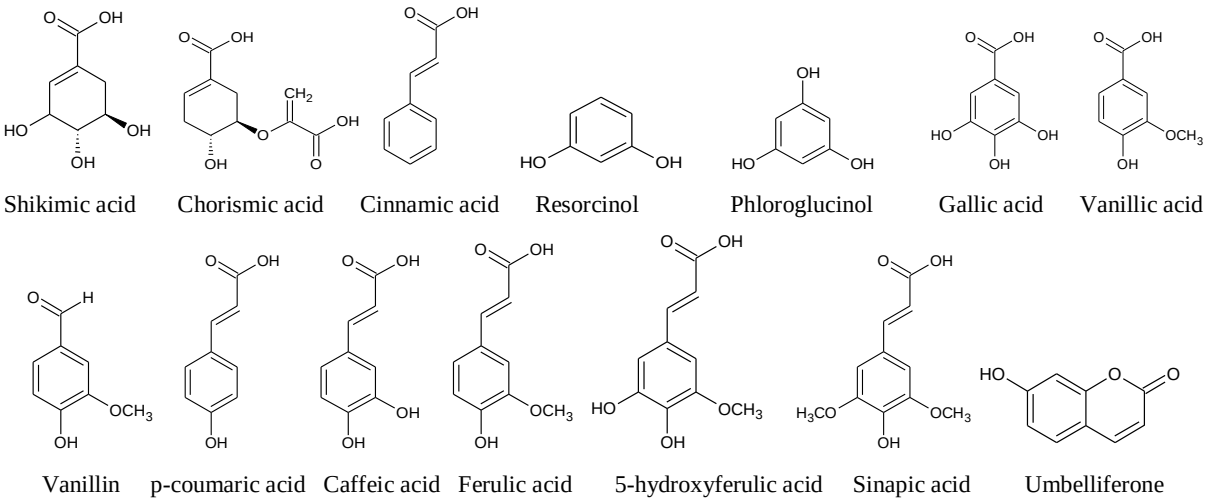

Figure I.4: Molecular structures of different simple phenols, acid phenols aldehydes, cinnamic acids and coumarins

I.2. Flavonoids

The flavonoid family is the biggest and the most studied group of polyphenols. Nowadays, more than 8000 flavonoids have been identified. They have a C6-C3-C6 skeleton (Fig. I.5).

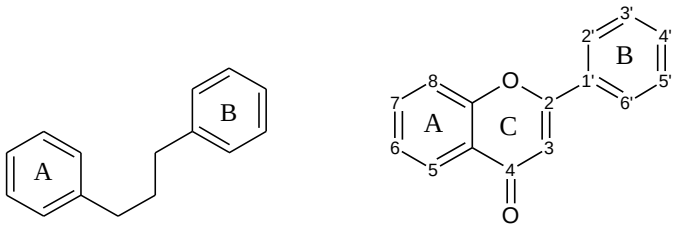

Figure I.5: General structure of of flavonoids (left), closed structure (right)

Depending on the nature of the basic structure, mainly (i) the nature of the hetero-ring (open or closed), (ii) the presence or absence of C2-C3 double bond and the carbonyl group (C=O) at C4 and (iii) the position of the B-ring (at C2 or C3), flavonoids are subdivided in various subclasses.

Most of flavonoids are substituted with an OH group at C5 and C7. The B-ring is often substituted at C3' and C4'. In plants, flavonoids are "free" or in their glycoside forms (i.e., conjugated with a sugar moiety).

I.2.1. Flavones

Flavones are characterized by the presence of a keto group at C4 and a 2,3-double bond (e.g., apigenin and luteolin) (Fig. I.6). The A-ring of the great majority of flavones is derived from phloroglucinol and the B-ring maybe substituted at C3', C4' and C5'.

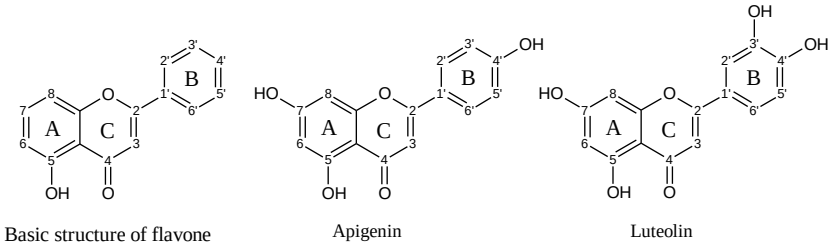

Figure I.6: Molecular structure of flavones

I.2.2. Flavonols

Flavonols are flavones in which possessing an OH group at C3 (Fig. I.7). Flavonols are widely distributed in plants. The most common flavonols are quercetin, kaempferol, and myricetin (Fig. I.7).

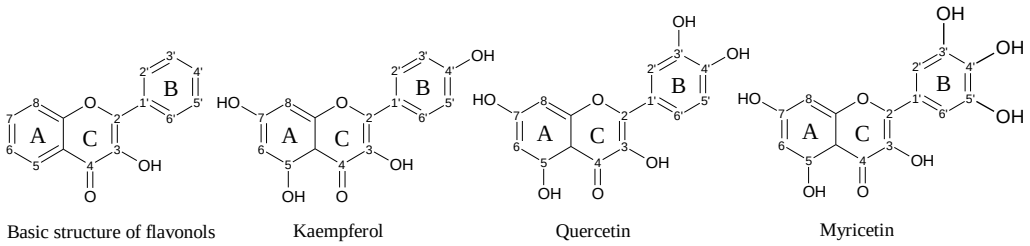

Figure I.7: Molecular structure of flavonols

I.2.3. Flavanones

Flavanones are characterized by the presence of a keto group at C4 and the absence of 2,3-double bond (2-phenyl-2,3-dihydropyran-4-one). Flavanones are isomeric with chalcones from which they are obtained synthetically and biosynthetically. Flavanones have a center of asymmetry at C2 giving two stereoisomers which is of crucial importance for some biological activities (mainly when ligand-receptor interactions are involved). The most common flavanones are naringenin, eriodictyol and hespertin (Fig. I.8).

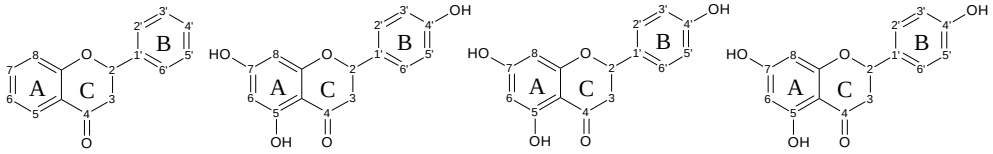

Figure I.8: Molecular structure of flavanones

I.2.4. Dihydroflavonols

Dihydroflavonols are flavanones substituted at C3 by an OH group (2-phenyl-3-hydroxy-2,3-dihydropyran-4-ones) (Fig. I.9). They exhibit two asymmetric carbons, C2 and C3, thus giving two couples of diastreoisomers. The most common flavanols are aromanderin, taxifolin and ampelopsin (Fig. I.9).

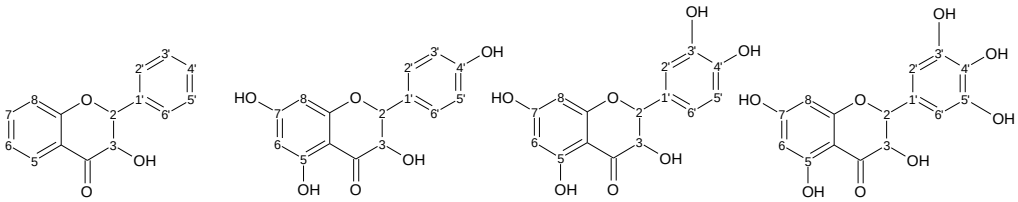

Figure I.9: Molecular structure of dihydroflavonols

I.2.5. Flavanols

Flavanols are characterized by the absence of the keto group at C4 and a 2,3- single bond (Fig. I.10). Two subclasses exist (i) flavan-3,4-diols and (ii) flavan-3-ols. Catechin and epicatechin are the most widely distributed flavan-3-ols, they are partly responsible for the beneficial effect of green tea. They are also combined with gallic acid to give epigallocatechin gallate or epicatechin gallate.

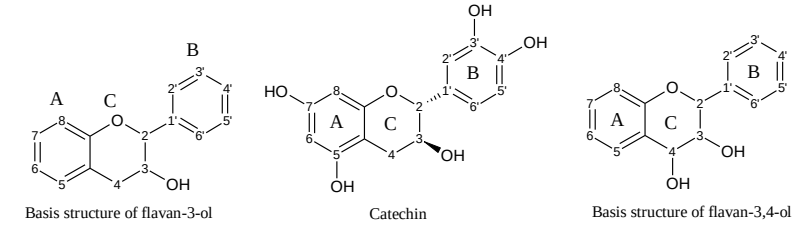

Figure I.10: Molecular structure of flavanols

I.2.6. Chalcones and dihydrochalcone

Chalcones (1,3-diaryl-2-propen-1-one) are the open-chain flavonoids in which the two aromatic rings are linked by a three-carbon α,β-unsaturated carbonyl system (Fig. I.11).

It should be noted that numbering in chalcones is reversed as compared to the other flavonoids i.e., the A-ring is numbered 1'-6' and the B-ring 1-6 (Fig. I.11).

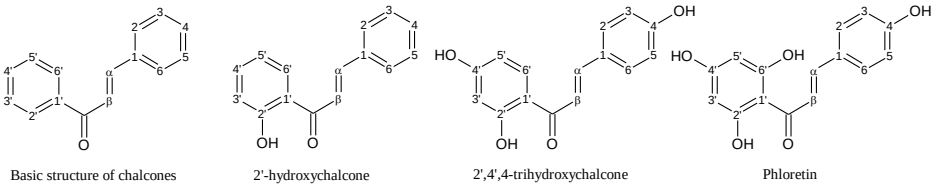

Figure I.11: Molecular structure of chalcones

The presence of a α,β-unsaturated bond and the absence of the central C-ring are two specific characteristics of chalcones, making these compounds chemically different from the other flavonoids.

Chalcone synthesis is catalyzed by the chalcone synthase. This compound is an intermediate of great importance in plant since it is the precursor of most of the other flavonoids. The transformation of chalcone to flavanone is catalyzed by the chalcone isomerase enzyme.

I.2.7. Isoflavones

In isoflavones the B-ring is substituted at C3 instead of C2 for the other flavonoids (e.g., genistein and daidzein) (Fig. I.12).

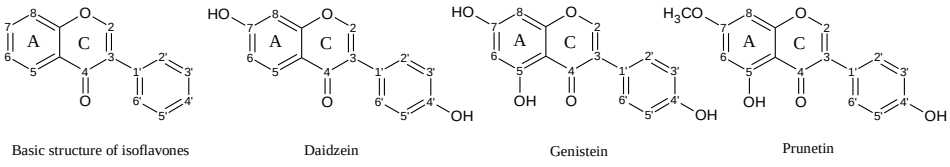

Figure I.12: Molecular structure of isoflavones

I.2.8. Anthocyanidins

Anthocyanidin skeleton is based on pyrylium cation (Fig. I.13). They are characterized by the presence of a cationic charge. Anthocyanidins are typically not found as free aglycons. Most of anthocyanidins are partly responsible for colour variation in fruits and flowers. The most common anthocyanidin are cyanidin, pelargonidin and delphinidine (Fig. I.13).

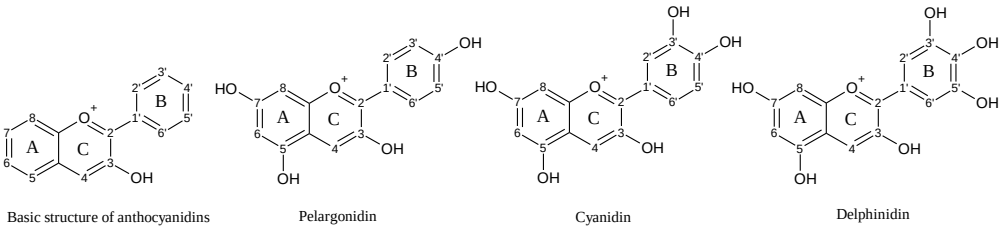

Figure I.13: Molecular structure of anthocyanidins

I.3. Other natural phenolic compounds

There exist a lot of other polyphenol subclasses derived from shikimates including lignans, flavonolignans, diarylheptanoids, stilbenoids and xanthones (Fig. I.14). Most of them are biologically active (e.g., antioxidant, anti-inflammatory). All these "small" polyphenols can dimerize (either naturally or synthetically). They thus can form bigger polyphenol compounds (oligomers and polymers). Oligomers and polymers of catechin derivatives (C4-C8, C4-C6 and C6-O-C7) are called tannins procyanidins and proanthocyanidins. They are widely distributed in grape and wine. They have been used over ages for their capacity to combine macromolecules like proteins for skin treatment. This property also explains astringency of wine (combination of tannins to the proteins of saliva).

Most of natural polyphenols (e.g., quercetin) are glycosilated (glycoside from) in fruit and vegetables. When they are absorbed by the organism they are easily deglycosylated in the small intestine. The corresponding aglycons are then conjugated (glucuronated, sulphated and methylated) at different positions to form metabolites that are naturally found in tissues. For instance, quercetin is known to be glucuronated preferentially at 3-OH (Fig. I.14). Methylation and sulphation occur preferentially at C3' (Fig. I.14).

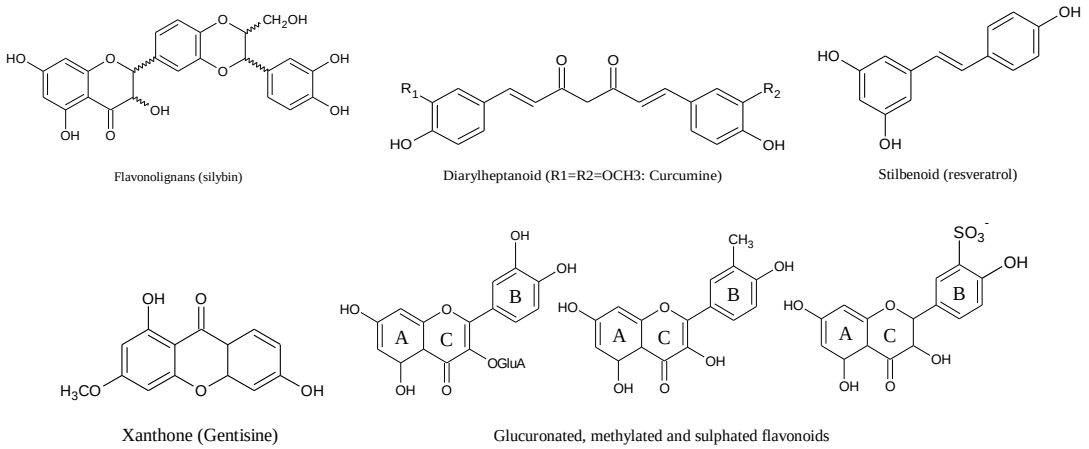

Figure I.14: Molecular structure of Flavonolignan, Diarylheptanoid, Stilbenoid, Xanthone, and Glucuronated, Methylated, Sulphated polyphenols

Recently a database has been built (www.phenol-explorer.eu) and that has systematically collected the content of polyphenols in 452 different foods and beverages.

II. Distribution of polyphenols

According to their solubility and their distribution, polyphenols can be divided into two categories: (i) the water-soluble polyphenols found e.g. in wine, tea, rice hulls, and fruit and (ii) fat-soluble polyphenols found e.g. in olive oil and sesame seeds. In the following section, we show the most important sources of polyphenols and/or interesting ones in views of their antioxidant and biological activities as tested in vivo.

Natural polyphenols are widely distributed in our daily diet i.e., fruit, vegetables, spices and various beverages (fruit juices, wines, tea, herbal tea …)

Originally, their concentration and presence in plants had been studied by phytochemists. However over the past 30 years natural polyphenols have gain back interest due to their numerous biological activities. Their distribution has been studied concomittently with various epidemiological studies, which was attributed to the beneficial effect of certain diets on human health.

Among these studies:

(i) the "French paradoxe*"* correlates the polyphenol content in wine (mainly red wine) with the decrease of the cardiovascular risk in the South west of France (where the consumption of food rich in saturated fat acid is relatively high) [11]. The regular (but moderate) intake of red

wine was correlated to the relatively low occurrence of cardiovascular disease as compared to 17 other countries [11].

The presence of quercetin and resveratrol derivatives appeared as the most important. Procyanidins may also participate in the decrease of cardiovascular diseases [12]. The main source of polyphenols in wine comes from the skin and seeds.

(ii) Renault *et al.* also studied the effect of beer *vs* wine in an eastern region of France [13]. They concluded that a moderate intake of both wine and beer may reduce the risk of cardiovascular death. This was partly attributed to the polyphenol content in both beverages.

(iii) Tea (Camellia sinensis) is consumed in many regions of the world. It is consumed as green tea or black tea, with or without sugar, milk, other plants, spices. It may be consumed either hot or fresh. Epidemiological studies demonstrated the beneficial effect of tea, mainly green tea, in the prevention of certain cancers (lung and stomach) [14-16] and cardiovascular diseases. Most of those beneficial effects are attributed to the presence of catechin in green tea. In black tea polyphenols are transformed by an enzyme (an oxidase) that is active during the fermentation process.

(iv) The Zutphen Study is an epidemiological study performed on a cohort of 552 men in the Netherlands. It correlated the presence of flavonoids in diets with the decrease of various diseases. About 28 vegetables, 12 fruits, and 9 drinks were evaluated for their concentration in quercetin, kaempferol, myricetin, apigenin, and luteolin. The main sources of flavonoids were showed to black tea (61%), onions (13%), and apples (10%). A significant inverse correlation was observed between the flavonoid intake and mortality. The most consumed flavonoid was quercetin (16.3 mg/day i.e., 63% of the total flavonoid supply), followed by kaempferol (8.2 mg/day i.e., 32%). According to Hertog [17], the human plasma concentration of quercetin is in the order of 1 µmol/L. At this concentration, quercetin protects Low Density Lipoproteins (LDL) from *in vitro* and *in vivo* oxidation. Consequently, it is probable that it has a protective effect against atherosclerosis [17]. Further information on the content of flavonoids in 28 vegetables and 9 fruits is given in reference [18].

(v) The olive tree (Olea Europa) grows especially throughout of the Mediterranean basin and olive oil is widely consumed in this area. The prevalence of cardiovascular pathologies is three times smaller in Spain than in England and this was partly attributed to the consumption of olive oil in Spain. The beneficial effect of this oil is explained by the biggest monounsaturated/polyunsaturated fatty acid ratio and a big concentration of polyphenols. The

major polyphenolic compound in olive oil is oleurophin which is present in the bark, the leaves and the olive fruit itself. It is a glucoside of elenolic acid esterified by 3,4-dihydroxyphenylethanol or hydroxytyrosol. By hydrolysis, oleuropin liberates hydroxytyrosol. There are also many other phenolic compounds, such as verbascoside (by hydrolysis verbascoside liberates caffeic acid and hydrotyrosol), rutin and luteolin. Most of polyphenols are etherified or esterified. Polyphenols of olive oil are very stable and weakly oxidized, mainly in virgin oil. Gutfinger selectively extracted phenols from four virgin olive oils and observed a spectacular fall of the oxidative stability of these oils [19]. This author obtained a linear relationship between the phenol content and oxidative stability.

Chimi and Cillard demonstrate that the phenolic compounds in olive oil are able to trap free radicals [20]. The trapping capacity depends on the phenol concentration, caffeic acid is able to trap free radicals at a concentration as low as 0.6 10^{-3} $mol.L^{-1}$, a concentration 10 times higher is required for the other. Léger *et al.* showed that polyphenols from olive are able to scavenge superoxide anion and to decrease its production in the cell [21].

(vi) Sesame is considered as the oldest oil plant. Sesame oil also offers a great resistance to oxidation, and this was thought to be due to the presence of sesamol [22-24]. The major antioxidant that protects sesame oil is sesaminol (Fig. I.15), which is present in substantial amount in sesame oil [25].

Sesaminol is fat soluble and thermostable [24]. In model systems based on corn oil, Osawa *et al.* showed that it is able to prevent the degradation of α-tocopherol [26]. Moreover, sesame oil contains γ-tocopherol [27], which is a particularly efficient antioxidant.

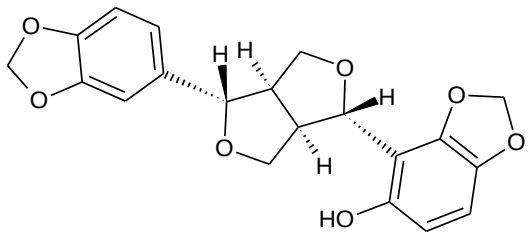

Figure I.15: Molecular structure of sesaminol

As a conclusion polyphenols (healthy polyphenols) are widely distributed in our daily diet and also in numerous beverages. Depending on their chemical structures they are present in fruit and vegetables (Tables I.1, I.2 and I.3) but also in various spices (e.g., sage, black pepper, nutmeg, rosemary, thyme, oregeus, melisse and seriette).

Table I.1: Total polyphenol content (mg/kg FW) in grape [28]

Tissue	% Fresh weight	Red	White
Skins	15	1800	900
Pulp	1	40	35
Juice	78	210	175
Seeds	6	3500	2800
Total		5600	3900

Table I.2: Typical levels of phenolics in red and white wine[a]

Phenol Class	White wine		Red wine	
	Young	Aged	Young	Aged
Nonflavonoids	154	130	165	60
Hydroxycinnamates	10	15	60	60
Benzoic acids	0	100	0	250
Hydrolyzable tannins (from oak)	0.5	0.5	7	7
Stilbens (resveratrol)	164.5	245.5	232	377
Total mg/L				
Flavonoids				
Flavonol monomers	25	15	200	100
Pronathocyanidins and condensed tannins	20	25	750	1000
Flavonols	-	-	100	100
Anthocyanins	-	-	400	90
Others	-	-	50	75
Total mg/L	45	40	1500	1365
Total all phenols	209.5	285.5	1732	1742

[a]Young means new wine < 6-months; not having aged or fermented in oak barrels.
Age implies~1 year white, ~ 2 years for red, and some oak barrel aging or other oak contact

Table I.3: Polyphenol Content of Selected Foods

Class and subclass	Representing compounds	Food or beverage	Quantity (mg)[a,b]
Flavonoids			
Flavonols	Quercetin	Olives	550
	Kaempferol	Onions	350
	Myricetin	Kale	320
		Leaf Lettuce	310
		Cranberry	250
		Cherry tomato	100
		Broccoli	100
		Apple juice	40
		Green tea	40
		Black tea	20
		Red wine	15
Flavanols	Catechin	Pear	250
	Epicatechin	Red wine	270
		Green tea	180
		White wine	35
		Apple	30
Flavones	Apigenin	Celery	130
	Luteolin	Olives	20
Flavanones	Hespertin	Grapefruit	500
	Hesperidin	Orange	500
Isoflavanes	Glabridin	Licorice root	500
Isoflavones	Genistein	Soybean	1500
	Daidzein	Soy nuts	1900
Phenolic acids and phenolics			
Hydroxycinnamic acids	Caffeic acid	Blueberry	2000
	Ferulic acid	Cherry, sweet	800
	Rosmarinic acid	Pear	600
	Carnosic acid	Apple	150
	Gingerol	Orange	100
	Hydroxytyrosol	Grapefruit	40
Hydroxycinnamic acids	Oleuropein	Ginger	
		Olive oil	
Phenolic acids and phenolics			
Hydroxybenzoic acids	Ellagic acid	Raspberry	60
	Gallic acid	Strawberry	50
		Grape juice, black	
		Grape juice, green	
Tannins			
Condensed	Catechin polymers	Red wine	25000
		White wine	240
		Apple juice	50
		Pomegranate	1200

[a]Quantities given are a total of all phytochemical included in the subclass, and not for individual phytochemicals
[b]Milligrams per kilogram, or milligrams per liter juice

III. Antioxidants activity of polyphenols

III.1. How to measure the antioxidant activity?

III.1.1.The DPPH method

To test the antioxidant activity of polyphenol compounds, several methods exist [29, 30]. One of the most famous methods is based on the use of the stable free radical 2,2-diphenyl-1-pycrilhydrazyl so-called DPPH (Fig. I.17). This is a stable radical in ethanol. The stability of DPPH is explained by the delocalization of the single electron over the radical, which avoid the dimerization or recombination as for most of other free radicals. This π-electron delocalization also explains the deep violet colour of DPPH in solution. It is characterized by an absorption band ranging from 517 to 520 nm, in methanol. In the presence of antioxidants as polyphenols (ArOH), DPPH is scavenged, its concentration decreases and the violet colour decreases, which can easily be measured.

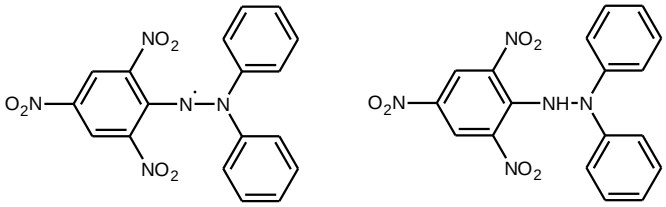

Figure I.17: Molecular structure of DPPH radical and its parent molecule

The use of DPPH to test the antioxidant capacity was introduced by Marsden Blois [31] and the test was optimized afterward [32-34].

The free radical scavenging capacity is evaluated by the IC_{50}, defined as the concentration of the substrate that causes 50% loss of the DPPH signal (colour) (i.e., that scavenge 50% of free radicals); the higher the antioxidant activity, the lower IC_{50}. This is a disadvantage particularly when results are presented graphically as a bar chart [35]. Because of its paramagnetic properties, DPPH also exhibits a characteristic ESR (Electron Spin Resonance) signal. The antioxidant capacity can also be measure as the capacity of the compound to decrease the ESR signal.

III.1.2. Measure of the anti-lipoperoxidation activity

The anti-lipoperoxidation is estimated as the capacity to inhibit the formation of oxidative products on lipids [36, 37]. Liposomes are oxidized by AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride), at ambient temperature, which normally leads to the formation of free radical $LOO^{\bullet}$ and other degradation products. The presence of these products is measured by their UV/visible signal at 233 nm.

III.2. Structure-antioxidant activity relationship of polyphenols

The antioxidant properties of polyphenols are related to the nature of their chemical structures [38-40]. Flavonoids are effective scavengers of hydroxyl, peroxyl, and superoxide anion radicals [41-43]. A number of *in vitro* studies have established the hierarchy of flavonoids in term of their antioxidant activities, which gives the corresponding structure–activity relationships [44, 45]. The activity is improved by:

(i) The presence of an ortho-dihydroxy structure in the B-ring (catechol moiety) as in quercetin (Fig. I.7). It has clearly been proved that the B-ring is the most important site for H-transfer and consequently for the antioxidant capacity. In contrast, the A-ring seems to be less important.

(ii) The presence of the 2,3-double bond also contributes to the antioxidant activity by increasing π-electron conjugation [40].

(iii) Hydroxyl group at position C3 in C-ring, is required for a maximal radical-scavenging potential. *In vitro* studies demonstrated that 3-OH contributes to the antioxidant potential, indeed, blocking the 3-OH group by a sugar moiety, as in rutin, or the absence of this group, as in luteolin, significantly decreases the activity [45]. However, the effectiveness of this group seems to be dependent on the presence of the 2,3-double bond and the C4 carbonyl group.

Theoretical calculations using quantum chemistry methods as density functional theory (DFT) or semi-empirical methods have been useful to elucidate and confirm the antioxidant structure-activity relationship of phenolic compounds [46-56].

A quantum-chemical study using both B3LYP and B3P86 functionals shed light on the reactivity of two flavonoids, quercetin and taxifolin, the corresponding dihydroflavonol of quercetin (Fig. I.18) [57]. This study focuses on the role of the 3-OH group and the correlation with the 2,3-double bond. Quercetin possesses all requirements to be a reference

phenolic antioxidant (ortho-OH groups in the B-ring, 2,3-double bond and 3-OH group). The antioxidant activity of taxifolin is twice lower than that of quercetin [39]. Theoretical results are compared and analyzed in terms of the O-H BDE (Bond Dissociation Enthalpy), the electron distribution in the singly occupied molecular orbital (SOMO) and the Mulliken spin density distribution in the radical species formed after hydrogen atom transfer from the polyphenol to the free radical. The analysis of the theoretical BDE values clearly confirmed the importance of the B-ring and the 3-OH group only when the 2,3-double bond is present like in quercetin. These results confirmed those obtained experimentally [45].

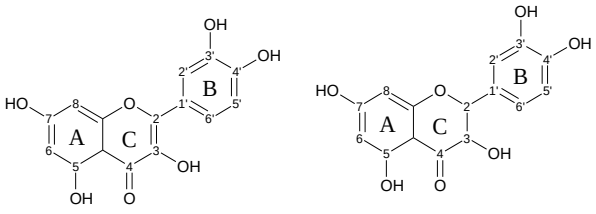

Figure I.**18**: Molecular structure of quercetin and taxifolin

The antioxidant activity of two flavonolignans (dehydrosilybin and silybin) (Fig. I.19) and their derivatives were studied in a joint experimental and theoretical study [58]. Dehydrosilybin is characterized by the presence of the 2,3-double bond and silybin is characterized by the absence of this double bond. Both flavonolignans were tested *in vitro* for their capacity to scavenge the stable DPPH free radical and the superoxide anion radical, and to inhibit the lipid peroxidation induced on microsome membranes. The experimental antioxidant activities were compared to the theoretical redox properties obtained by quantum-chemical calculations of different parameters including BDE (Bond Dissociation Enthalpy) of the OH groups. The importance of the 3-OH group in the presence of the 2,3-double bond and the role of catechol moiety were confirmed. The free radical scavenging capacity of dehydrosilybin with the 2,3-double bond is much higher than for the silybin derivatives, which do not possess this double bond. DPPH scavenging capacity indicates that 3-OH is the most important group involved in this activity. The 20-OH group also seems to influence the antioxidant activity while the other two groups (7- and 5-OH) appear to give only a small contribution. 19-Nor-dehydrosilybin (Fig. I.19) exhibits a much higher antioxidant activity compared to the other compounds with DPPH and superoxide scavenging capacities very

closed to that of quercetin. This is attributed to the catechol moiety of the E ring (Fig. I.19). This compound is a better inhibitor of lipid peroxidation than quercetin, probably because it possesses the same redox capacity and it is more lipophilic.

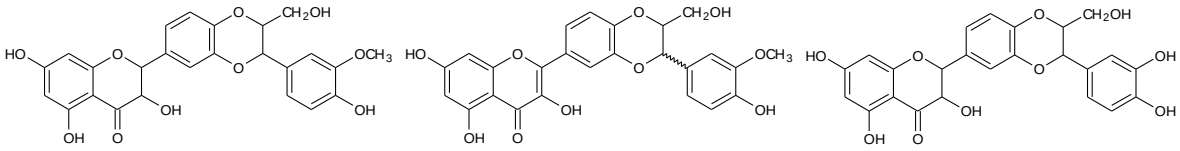

Figure I.**19**: Molecular structure of silybin, dehydrosilybin and nor-dehydrosilybin

It should be noted that no linear relationship was found between the IC_{50} of DPPH scavenging and the IC_{50} of lipid peroxidation inhibition. First, the mode of action for DPPH and $ROO^{\bullet}$ scavenging could be different (due to the different redox properties of those two free radicals). Then, DPPH can undergo different types of reaction including adduct formation, while lipid peroxidation is strongly influenced by the lipophilicity of the compounds, which could distort the experimental results on the pure scavenging (or inhibition) capacity. Therefore, the correlation between those two tests is not necessarily good.

Kozlowski *et al.* [59] studied the antioxidant activity of a series of chalcones; they compared the results obtained by the use of quantum chemical calculations and experimental tests of the antioxidant activity in regard to the capacity of these chalcones to scavenge the DPPH (2,2-diphenyl-1-pycril-hydrazyl) free radical. The obtained results confirmed the role of the catechol moiety in the B-ring to explain the redox properties of chalcones. The role of the other OH groups of chalcones, especially the 6'-OH was discussed. This group is involved in the anti-estrogenic action (which correlates with the DPPH scavenging activity) of chalcones. Its role in the redox capacity is weaker than that of the OH groups of the catechol moiety; however, the BDE in the presence of the α,β-double bond is sufficiently low to enable hydrogen transfer to a group with a relatively high H atom affinity (DPPH and ROO• radicals and, perhaps, an estrogen receptor).

IV. Mechanism of action of polyphenols

It is well-established that the redox reactivity of phenolic compounds (ArOH) to scavenge free radicals can follow four chemical pathways.

IV.1. Proton Coupled-Electron Transfer PC-ET

In this mechanism, the antioxidant (ArOH) scavenges free radicals (e.g., peroxyl radical $ROO^{\bullet}$) by the transfer of an H-atom of the OH groups to the free radical:

$$ArOH + ROO^{\bullet} \rightarrow ArO^{\bullet} + ROOH \qquad \text{(reaction I. 16)}$$

The produced phenoxyl radical ($ArO^{\bullet}$) can be stabilized by further H-atom transfer and the formation of quinones, or by reacting with another radical, including another phenoxyl radical, thereby interrupting the initiation of a new chain of reaction.

The PC-ET mechanism corresponds to the homolytic dissociation of OH bonds of polyphenolic compounds. This reaction can occur on each OH group of the phenolic compound (ArOH) depending on the BDE of the OH group and the enthalpy of reaction (reaction I.16).

The BDE is an intrinsic thermodynamic parameter of a given OH group in a phenolic compound while enthalpy of reaction depends on the radical reacting with the polyphenol compound. The lower the BDE, the easier the O-H bond breaking and the more important its role in the antioxidant reactivity. To be active on a given free radical, the OH group must exhibit a negative enthalpy, so that reaction is exothermic.

IV.2. Electron transfer-Proton transfer (ET-PT)

This mechanism is a two-step mechanism (reaction I. 17), in which the first step is an electron transfer from the polyphenol to the free radical and the second step a proton transfer.

$$ArOH + ROO^{\bullet} \rightarrow ArOH^{+\bullet} + ROO^{-} \rightarrow ArO^{\bullet} + ROOH \qquad \text{(reaction I. 17)}$$

ET-PT mechanism is governed by the electron transfer capacity, in other words, the ionization potential (IP). The second step of this reaction is the heterolytic O-H bond dissociation, which is strongly exothermic for phenolic compounds [60, 61]. In order to

estimate the contribution of the ET-PT mechanism of polyphenol compounds, we have to calculate the IP for all the compounds. The presence or absence of the 2,3-double bond appears to be the most important chemical parameter that influences IP. The lowest IP is obtained for compounds having the 2,3-double bond. The solvent strongly influences the enthalpy of reaction of the first step which leads to the conclusion that the solvent must be taken into account, in order to obtain a good description of the redox properties of polyphenolic compounds. The ET-PT mechanism can play a secondary role in some reaction. However, the corresponding enthalpy of reaction for the first step values for the DPPH and $ROO^{\bullet}$ radicals are too high to consider the ET-PT mechanism as the major process. Indeed, the ET mechanism gives endothermic reactions with DPPH as well as with peroxy radicals.

IV.3. Sequential Proton Loss Electron Transfer (SPLET)

In the SPLET mechanism (reaction I. 18) [62-64], a proton is first lost, thereby allowing electron transfer. This mechanism is favoured in specific pH conditions. The final products are the same than for PC-ET and ET-PT.

$$\begin{aligned} &ArOH \rightarrow ArO^- + H^+ \\ &ArO^- + ROO^{\bullet} \rightarrow ArO^{\bullet} + ROO^- \quad \text{(reaction I. 18)} \\ &ROO^- + H^+ \rightarrow ROOH \end{aligned}$$

IV.4. Adduct formation (AF)

Adduct formation is also a free radical scavenging mechanism (reaction I. 19). This may lead to the formation of relatively stable compounds [65].

$$ArO^- + ROO^{\bullet} \rightarrow [ArO-R]^{\bullet} \rightarrow \text{Stables metabolites} \quad \text{(reaction I. 19)}$$

This mechanism is relatively specific and is mainly observed in solution rich in reactive species e.g., radiolysis solutions.

Polyphenols and colour

Plants offer a wide variety of colours. Leaves, petals, roots, fruits of plants may possess all the colours that can be viewed by human vision (from red to purple). They may also exhibit UV colour variation visible for some insects and other animals. The colour of the different plant organs are mainly attributed to the accumulation of flavonoid derivatives in vacuoles. After their formation in plant as secondary metabolites they are transferred to the vacuole by enzyme controlled process. There, the coloured compounds may accumulate and remain chemically stable during a long period. They can also aggregate by π-stacking interactions.

Anthocyanins are considered as the most important flavonoid pigments (Fig. I.13). They are water-soluble pigments which colour plant with all the possible colours, depending on their chemical structures. Widely accumulated in veins of leaves (e.g., tobacco, potatoes), flowers (e.g., orchid, roses), fruit (e.g., tomato, kiwi, aubergine, berry, grape) and (fermented) fruit juices (e.g., wine). The genetic pathway that allows production and accumulation of anthocyanins has widely been studied to change the colour of e.g., tomatoes (orange and purple).

Chalcones usually give yellow to orange colours to the plant organs in which they are located. For the other flavonoids it strongly depends on the chemical structures. They are usually yellow to colourless (i.e., absorb UV) but slight differences in the supramolecular structure (e.g., presence of sugar moieties, π-staking interaction) may change their colour.

The real nature of light and colour sensation remained unknown for a long time. Neither the greek & roman philosophers nor the scientists of the middle ages and renaissance did exactly know what was light. In 1666, Isaac Newton performed the first experiment on light showing that the white light from sun is actually a mixture of red, orange, green, blue and purple (i.e., all the colours that can be distinguished by the human eye in the rainbow).

Table I.4: The colours of the visible light spectrum

Colour	Wavelength interval (nm)
red	700–635
orange	635–590
yellow	590–560
green	560–490
blue	490–450
violet	450–400

Isaac Newton introduced the notion of spectrum. Spectrum means “ghost” in latin because the colours that compose white light is immaterial. But the colours exist and can be viewed by using experiments or in nature (rainbow, interferences of thin oil layer at the surface of water). When light interacts with an object or living organism (e.g., fruit or flowers) wavelengths are absorbed, transmitted, reflected or diffused. That is a petal appears (to our eye and brain) blue if it contains molecules that absorb red light.

Due to extended π-electron conjugation, the π-π* transitions of flavonoids are spectroscopically active in the UV/visible range. Depending on the effective conjugation length some are coloured (yellow, blue, and red), while others are colourless.

Many researches focused on the UV/visible absorption spectra of flavonoids [66-69]. UV/visible spectroscopy has become a major technique for the structure analysis of flavonoids besides to the other methods of analysis (e.g., infrared, NMR). To measure a UV/visible spectrum only a small amount of pure materials is required, a single flavonoid spot on a chromatogram paper yields the useful UV/visible spectra information.

The UV/visible spectrum of most of the flavonoids consists of two major absorption maxima, one in the range 240-285 nm (band II) and the other in the range 300-400 nm (band I). Band II is usually assigned to the A-ring and band I to the B-ring [70].

For example, flavones and flavonols with OH groups in the A-ring, but not in the B-ring, tend to give spectra in methanol with a pronounced band II and a weak band I. On the contrary, for molecules with OH groups in the B-ring, band I is more pronounced and appears at higher wavelengths (red shifted).

Isoflavones, flavanones and dihydroflavonols all give similar UV/visible spectra as a result of the lack in conjugation between the A- and B-rings. They differed from flavones and flavonols by exhibiting intense band II absorption and only a shoulder or low intensity peak for band I.

Anthocyanidins and their glycosides have a maximum absorption band in the range 465-550 nm, band II being represented by a less intense peak in the 270-280 nm region.

For all flavonoids, the number of OH groups in the B-ring leads to a bathochromic shift (i.e. towards longer wavelength) of band I. The position of band I can also be a guide to substitution in the A-ring as showed by Jurd and Harborne [71, 72]. Methylation and glycosylation of both A and B rings generally produce small hypsochromic shifts (i.e. towards shorter wavelengths).

The chalcones are characterized by the presence of an open C-ring. Their UV/visible spectra show a major band I and a relatively minor band II.

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Chapter 2: Basic concepts in theoretical chemistry

The Hartree-Fock Approximation

I. Introduction

I.1. Schrödinger Equation

Quantum chemistry aims at rationalizing the chemical behaviour of molecular systems (reactivity, spectroscopic signature…). A molecule is a group of atoms i.e., electrons and nuclei. The Schrödinger equation describes the motion of electrons and nuclei within the quantum mechanic formalism [1]. For a molecular system which consists of N_A nucleus and n electrons, the time-independent non-relativistic Schrödinger equation is given by:

$$\widehat{\mathcal{H}}\Psi(\mathrm{r},\mathrm{R}) = \mathrm{E}\Psi(\mathrm{r},\mathrm{R}) \qquad \text{(II. 1)}$$

where $\widehat{\mathcal{H}}$ is the Hamiltonian operator, $\Psi(\mathrm{r},\mathrm{R})$ the wave function of the system, r and R are the electronic and nuclear coordinates, respectively. E is the energy of the molecular system. $|\Psi(\mathrm{r},\mathrm{R})|^2$ represents probability density. The developed expression of the Hamiltonian operator in atomic units is:

$$\widehat{\mathcal{H}} = -\frac{1}{2}\sum_{\mathrm{i}=1}^{\mathrm{N}}\nabla_{\mathrm{i}}^2 - \frac{1}{2}\sum_{\mathrm{A}=1}^{\mathrm{N_A}}\frac{1}{\mathrm{M_A}}\nabla_{\mathrm{A}}^2 - \sum_{\mathrm{i}=1}^{\mathrm{N}}\sum_{\mathrm{A}=1}^{\mathrm{N_A}}\frac{\mathrm{Z_A}}{\mathrm{r_{iA}}} + \sum_{\mathrm{i}=1}^{\mathrm{N}}\sum_{\mathrm{j}>i}^{\mathrm{N}}\frac{1}{\mathrm{r_{ij}}} + \sum_{\mathrm{A}=1}^{\mathrm{N_A}}\sum_{\mathrm{A}>B}^{\mathrm{N_A}}\frac{\mathrm{Z_A Z_B}}{\mathrm{R_{AB}}} \qquad \text{(II. 2)}$$

The first two terms are associated to the kinetic energies of electrons and nucleus, respectively ($\mathrm{T} = \mathrm{T_e} + \mathrm{T_N}$). The other three terms are the attractive potential energy between electrons and nucleus ($\mathrm{V_{eN}}$) and the repulsion potential energy between electrons ($\mathrm{V_{ee}}$) and nuclei ($\mathrm{V_{NN}}$), respectively. $\mathrm{M_A}$ and $\mathrm{Z_A}$ are the mass and atomic number of atom A, respectively.

I.2. Born-Oppenheimer Approximation

There are too many variables to solve truly the Schrödinger equation for big molecular systems. Various approximations have been proposed over the past century. The primary is the one proposed by Born and Oppenheimer [2]. They suggested to divide the problem into two problems.

The first concerns the motion of electrons. In this case, the kinetic energy of nucleis is neglected since nuclei are much heavier, and thus slower, than electrons. The motion of electrons is viewed in a field of fixed nucleus-charges. The electronic Schrödinger equation for a molecular system is given by:

$$\hat{\mathcal{H}}_e \Psi_e(r, R) = E_e \Psi_e(r, R) \qquad \text{(II. 3)}$$

where $\Psi_e(r, R)$ is the electronic wave function in which R is a parameter (not a variable), E_e is the electronic energy and $\hat{\mathcal{H}}_e$ is the electronic Hamiltonian operator given by:

$$\hat{\mathcal{H}}_e = -\sum_{i=1}^{N} \frac{1}{2} \nabla_i^2 - \sum_{i=1}^{N} \sum_{A=1}^{N_A} \frac{Z_A}{r_{iA}} + \sum_{i=1}^{N} \sum_{j>i}^{N} \frac{1}{r_{ij}} \qquad \text{(II. 4)}$$

The total energy of the molecular system is the sum of the electronic energy E_e and a constant which presents the nuclear repulsion term:

$$E = E_e + \sum_{A=1}^{N_A} \sum_{B>A}^{N_A} \frac{Z_A Z_B}{R_{AB}} \quad \text{(II. 5)}$$

This first approximated solution gave rise to all the quantum chemistry methods.

The second problem proposed by the Born and Oppenheimer approximation treats explicitly the motion of nuclei. In this case electrons are considered implicitly as an average field; the problem is not quantum but classical. It is treated with classical mechanics and gave rise to all the Molecular Mechanics methods.

I.3. Variational principle for the ground state

To determine the wave function $\Psi_0(r, R)$ and the energy E_0 of the ground state, we can apply the variational principle. In the case of the Schrödinger equation the variational principle states that each approximated wave function $\Psi(r, R)$ gives an energy value higher than that of the ground state E_0. In other terms to find the true wave function $\Psi_0(r, R)$, the energy must be minimized:

$$E_0 = \min_{\psi} E[\psi] \qquad \text{(II. 6)}$$

II. The Hartree-Fock Approximation

II.1. Slater Determinant

In molecular orbital theory, a single electron is described by a spin-orbital wave function $\chi_i(x_i)$, which is the product of a spatial orbital $\phi_i(r_i)$ and a spin eigenfunction $\sigma(\omega_i) = \alpha(\omega)$ or $\beta(\omega)$ corresponding to spin up and down, respectively:

$$\chi_i(x_i) = \phi_i(r_i)\sigma(\omega_i) \qquad \text{(II. 7)}$$

For N electrons (polyelectronic systems), the total wave function is given by a Slater determinant [3]:

$$\Psi = \frac{1}{\sqrt{N!}} \begin{vmatrix} \chi_1(x_1) & \chi_2(x_1) & \chi_3(x_1) & \cdots & \chi_N(x_1) \\ \chi_1(x_2) & \chi_2(x_2) & \chi_3(x_2) & \cdots & \chi_N(x_2) \\ \chi_1(x_3) & \chi_2(x_3) & \chi_3(x_3) & \cdots & \chi_N(x_3)) \\ & & \cdots & & \\ \chi_1(x_N) & \chi_2(x_N) & \chi_3(x_N) & \cdots & \chi_N(x_N)) \end{vmatrix} \qquad \text{(II . 8)}$$

where $\frac{1}{\sqrt{N!}}$ is the normalization factor. The total energy that corresponds to such a Slater determinant is given by:

$$E = \sum_{i=1}^{N} H_i + \sum_{i}^{N} \sum_{j>i}^{N} (J_{ij} - K_{ij}) + \ H_0 \qquad \text{(II. 9)}$$

where H_i is the kinetic and potential energy of each electron moving in the field of nuclei:

$$I_i = \int \chi_i^* (x_1)\left[\hat{h}_i\right]\chi_i(x_1)d\tau_{x_1} \qquad \text{(II. 10)}$$

J_{ij} is the classical coulomb interaction potential between the electrons in the spin-orbitals i and j:

$$J_{ij} = \int\int \chi_i^*(x_1)\,\chi_j^*(x_2)\frac{1}{r_{12}}\chi_i(x_1)\chi_j(x_2)d\tau_{x1}d\tau_{x2} \qquad \text{(II. 11)}$$

K_{ij} is the non-classical exchange interaction potential:

$$K_{ij} = \int\int \chi_i^*(x_1)\chi_j^*(x_2)\frac{1}{r_{12}}\chi_i(x_2)\chi_j(x_1)d\tau_{x1}d\tau_{x2} \qquad \text{(II. 12)}$$

It physically corresponds to the motion due to correlation between electrons with the same spin (only due to the spin and not the charge). This is related to the Pauli principle which state that electrons with the same spin cannot (i) have the same energy and (ii) be at the same location.

H_0 is a constant which presents the repulsion potential between nuclei.

II.2. Hartree-Fock Approximation

The essence of the Hartree-Fock approximation is to replace the complicated many-electron problem by a one-electron problem in which electron-electron repulsion V_{ee} is treated in an average way [4, 5]. The electron does not see all the different electrons separately, but see a potential field which is due to the other electrons.

The Hartree-Fock energy is obtained by the summation over all the i spin-orbitals:

$$E_{HF} = \sum_{i=1}^{n}\varepsilon_i - V_{ee} \qquad \text{(II. 13)}$$

where V_{ee} is the total electron-electron repulsion energy and ε_i is the energy of the i^{th} molecular orbital given by:

$$\hat{f}_i\psi_i = \varepsilon_i\psi_i \qquad \text{(II. 14)}$$

where f_i is the one-electron Fock operator:

$$\hat{f}_i = H_i + \sum_{j=1}^{N} (J_{ij} - K_{ij}) \quad \text{(II. 15)}$$

The solution of the Hartree-Fock equation must proceed iteratively by the use of a SCF (Self-consistent-field) procedure. The starting wave function is a Slater determinant (chosen as a trial wave function), it helps to recalculate a better Fock operator, which in turn give a better wave function, until a self-converge is reached.

The Hartree-Fock equation can be solved using the RHF (restricted Hartree-Fock) approach for closed-shell systems (even number of electrons). For open-shell systems (odd number of electrons in their outer shells), either a ROHF (restricted open-shell Hartree-Fock) or an UHF (unrestricted open-shell Hartree-Fock) can be used.

In the RHF approach, electrons with α and β-spins are forced to occupy the same spatial orbitals $\phi_i(r_i)$. The RHF energy for a closed shell system is:

$$E_{HF} = 2\sum_{i=1}^{N/2} I_i + \sum_{i}^{N/2}\sum_{j>i}^{N/2} (2J_{ij} - K_{ij}) + H_0 \quad \text{(II. 16)}$$

In UHF, no restriction are imposed on the spatial nature of orbitals $\{\phi i\}$. An unrestricted set of spin-orbitals has the form:

$$\chi_i = \phi_i^{\alpha}\alpha(\omega) \quad \text{(II. 17)}$$

$$\chi_{i+1} = \phi_i^{\beta}\beta(\omega) \quad \text{(II. 18)}$$

UHF is especially used for system with an odd number of electrons (e.g., radicals).

II.3. The Roothaan-Hall equations

The direct solution of the Hartree-Fock equation is not practical. Roothaan [6] and Hall [7] proposed to develop each molecular orbital as a linear combination of atomic orbitals (LCAO-MO) (φ_μ):

$$\psi_i = \sum_{\mu=1}^{m} c_{\mu i}\varphi_\mu \qquad \text{(II. 19)}$$

The insertion of equation (II. 19) in the Hartree-Fock equation, leads to the Hartree-Fock Roothaan equations (matrix form):

$$\sum_{\nu} F_{\mu\nu}C_{\nu i} = \sum_{\nu} \varepsilon_i S_{\mu\nu}\, C_{\nu i} \qquad \text{(II. 20)}$$

where $F_{\mu\nu}$ is the element of the Fock matrix is, is given by:

$$F_{\mu\nu} = \int \varphi_\mu^* f_i \varphi_\nu dr_i \qquad \text{(II. 21)}$$

where

$$f_i = h_i + \sum_{j=1}^{N/2} 2J_j(r_i) - K_j(r_i) \qquad \text{(II. 22)}$$

$S_{\mu\nu}$ is the overlap integral between two atomic orbitals φ_μ and φ_ν:

$$S_{\mu\nu} = \int \varphi_\mu^* \varphi_\nu dr_i \qquad \text{(II. 23)}$$

II.4. Correlation Energy

The Hartree-Fock approximation does not take into account the electron correlation between electrons, and the corresponding energy. The energy obtained from the Hartree-Fock approximation is not exact. The error is called the correlation energy E_{corr}:

$$E_{corr} = E_{exact} - E_{HF} \qquad \text{(II. 24)}$$

where E_{exact} is the exact energy.

It is so-called “correlation energy” because the missing energy comes from the different correlation motions of electrons (e.g., correlation of electrons with anti-parallel spin).

II.5. Electron Density

The electronic density $\rho(r)$ is the number of electron per unit of volume in a given state. It is given by:

$$\rho(r_1) = N \int \cdots \int |\Psi(x_1, x_2, \ldots, x_N)|^2 \, dx_2 \ldots dx_N \quad (II.25)$$

The integration over the whole space is the total number of electrons (N):

$$\int \rho(r) dr = N \quad (II.\ 26\ )$$

(For atoms in the ground state, the electronic density decreases monotonically away from the nucleus [8]. For molecules, at first sight, densities look like superposed atomic densities; on closer inspection (experimental or theoretical), modest build-ups of density are seen in the bonding regions.)

III. Basis Sets

The choice of the basis set plays a crucial role to improve the accuracy of calculation.

III.1. Slater Orbitals functions

In order to solve the Schrödinger equation, a mathematical expression of molecular orbitals must be given. In LCAO (Linear Combination of Atomic Orbital) theory, MOs ψ_i are written as a linear combination of atomic orbital φ_μ (Slater Type Orbital):

$$\psi_i = \sum_{\mu=1}^{m} c_{\mu i} \varphi_\mu \qquad (II.27)$$

III.2. Contracted Gaussian functions

The Slater Type Orbital φ_μ can in turn be developed as a linear combination of Gaussian functions (g_i).

$$\varphi_\mu = d_1 g_1 + d_2 g_2 + d_3 g_3 + \cdots \qquad (II.28)$$

A contraction Gaussian function has the form:

$$\varphi_\mu^{CGF}(r - R_A) = \sum_{p=1}^{L} d_{p\mu} g_p(\alpha_{p\mu}, r - R_p) \qquad (II.29)$$

$\alpha_{p\mu}$ and $d_{p\mu}$ are the contraction exponents. L is the length of the contraction. For example, the normalized Gaussian primitive functions for 1s is given by:

$$g_{1s}(\alpha, r) = (8\alpha^3/\pi^3)^{1/4} e^{-\alpha r^2} \qquad (II.30)$$

III.2.1. Minimal Basis Sets STO-LG

The original basis sets used within the Hartree-Fock formalism were the STO-LG basis sets, in which each contraction is developed by L primitives ($1 \leq L \leq 6$). The most famous and used basis set of this type was the STO-3G [9-11]. These basis set weakly reproduced the experimental data and other refinements were developed over the past decades.

III.2.2. Double-Dzeta Basis Set

The mathematical expression of the double-dzeta basis sets is given by two contractions rather than one. For example, the 6-31G basis set uses one contraction to describe the inner shell AOs while the valence AOs are described by two contractions. For the inner shell MOs the unique contraction is developed by six primitives. For the valence AOs, the former contraction is developed by three primitives and the latter by only one primitive. This mathematical description allows more flexibility in the description of AOs. Following the same idea there exists the triple-dzeta basis sets.

III.2.3. Polarized Basis Set

To improve accuracy, polarization functions were also used. It consists in adding e.g., p-type and d-type contraction on H-atoms and heavy atoms, respectively.

III.2.4. Diffuse Function

The addition of diffuse function allows to enhance the description far from the nuclei. Such basis sets are quoted with the '+' and '++' for heavy atoms and H-atoms, respectively (e.g., 6-31+G(d,p) and 6-311++G(d,p)).

Density Functional Theory

I. Introduction

The Hartree Fock approximation is sometimes really good but has its limitations. The restricted HF method cannot describe the dissociation of molecules into open-shell fragments. Example: For phenol ^{HF}BDE = 49.5kcal/mol while the experimental BDE is about 87kcal/mol! What is missing is the correlation energy.

To take into account the electronic correlation many correlated methods have been developed for molecular calculations. Various post-HF methods have been developed, including those based on the Configuration Interaction approach and multi-reference methods, the Møller-Plesset perturbation theory (MP2, MP4…), Multi-configurational self-consistent field approaches (CASSCF, CASPT2…), coupled-cluster methodes. Most of these post-HF methods allow to reach a very good accuracy, nonetheless the computational time is dramatically increased and only relatively small molecular systems can be studied.

The density functional theory (DFT) has been developed to take into account the correlation correction. DFT appears as a good balance between accuracy and computational cost, allowing to treat much larger systems than with post-HF.

The original idea of DFT is to replace the complicated N-electron wave function $\Psi(x_1, x_2 \dots x_N)$ by the electronic density ρ(r) as a much simpler variable:

$$\rho(r_1) = N \int \int \cdots \int |\Psi(x_1, x_2, \dots x_N)|^2 dx_2 \cdots dx_N \quad \text{(II. 31)}$$

The wave function is something very complex and actually this is just a mathematical object, without physical reality. The fact that Ψ is written as a Slater determinant gives a sort of « physical meaning », as Ψ is built with the one electron orbitals. Nonetheless, Ψ is definitely not an interpretable. It can just give an interpretable as a response to an operator (e.g., $H\Psi = E\Psi$). In that sense, ρ(r) is a much interesting variable since it is directly related to a physical meaning.

An electronic system is entirely described by:

$$\hat{H} = \sum_{i=1}^{N} -\frac{1}{2}\nabla_i^2 + \sum_{i=1}^{N} v_{ext}(r_i) + \sum_{j>i}^{N} \frac{1}{r_{ij}} \qquad \text{(II. 32)}$$

in which the first term is the operator "kinetic energy", the last represents the electron-electron interactions, and the second one is the electron-nuclei electrostatic interaction (i.e.,$\sum_{A}^{N_A} \frac{Z_A}{r_{iA}}$) + any other external potential (e.g., electroc field).

For a N-electron system, N and v_{ext} completely fixes $\hat{H}$ and thus all properties for the ground state. The idea of DFT is to replace those two parameters by ρ(r).

II. The Hohenberg-Kohn Theorems

Two theorems were proposed by Hohenberg and Kohn [12].

II.1. The First Hohenberg-Kohn Theorem

In their paper, Hohenberg and Kohn [12] stated that "the external potential $v_{ext}(r)$ is (to within a constant) a unique functional of ρ(r), since, in turn $v_{ext}(r)$ fixes $\hat{H}$ we see that the full many particle ground state is a unique functional of ρ(r)". In other words, the electronic density of a system, in its fundamental state, totally determines the external potential. As ρ also determines the number of electrons N, ρ also determines the wave function of the fundamental state. As a consequence this determines all the electronic properties of the fundamental state, such as the kinetic energy $T[\rho_0]$, the potential energy $V[\rho_0]$ and the total energy $E_0 = E[\rho_0]$, which is given by:

$$E_0[\rho_0] = T[\rho_0] + E_{ee}[\rho_0] + V_{ext}[\rho_0] \quad \text{(II. 33)}$$

V_{ext} is the external potential functional, which is, in the absence of an external perturbation, the interaction between nuclei and electrons $V_{eN}[\rho_0]$, which is given by:

$$E_{Ne}[\rho_0] = \int \rho_0(r) V_{Ne}\, dr \qquad \text{(II. 34)}$$

The total energy can thus be written:

$$E_0[\rho_0] = T[\rho_0] + E_{ee}[\rho_0] + E_{Ne}[\rho_0] = F_{HK}[\rho_0] + E_{Ne}[\rho_0] \qquad \text{(II. 35)}$$

in which F_{HK} is the Hohenberg-Kohn functional:

$$F_{HK}[\rho] = T[\rho] + E_{ee}[\rho] = \langle\psi|\hat{T} + \hat{V}_{ee}|\psi\rangle \qquad \text{(II. 36)}$$

where $T[\rho]$ is the kinetic energy and $E_{ee}[\rho]$ is the electron-electron repulsion energy which can be developed as follows:

$$E_{ee}[\rho] = \frac{1}{2}\iint \frac{\rho(r_1)\rho(r_2)}{r_{12}} dr_1 dr_2 + E_{ncl}[\rho] = J[\rho] + E_{ncl}[\rho] \qquad \text{(II. 37)}$$

where $J[\rho]$ is the classical repulsion between electrons and $E_{ncl}[\rho]$ is the non-classical contribution that take into account the other electron-electron interactions including the exchange and correlation corrections.

II.2. The Second Hohenberg-Kohn Theorem: Variational Principle

In the second theorem, Hohenberg-Kohn stated that, from the Hohenberg-Kohn functional $F_{HK}[\rho]$, the ground state energy (lowest energy) is obtained if and only if the input density is the true ground state density ρ_0. In other word, from any trial density $\tilde{\rho}(r)$ associated to an external potential v_{ext}:

$$E_0 \leq E[\tilde{\rho}] = T[\tilde{\rho}] + E_{Ne}[\tilde{\rho}] + E_{ee}[\tilde{\rho}] \qquad \text{(II. 38)}$$

This is the variational principle for $E[\rho]$.

To summarize all electronic properties of a molecular system defined by an external potential v_{ext} are determined by the ground state density ρ_0. In particular the ground state energy associated to a density ρ can be obtained by the functional $\int \rho(r) V_{Ne} dr + F_{HK}[\rho]$. This functional reaches a minimum if and only if the input density is the true ground state density:

$$\tilde{\rho}(r) \equiv \rho_0(r) \qquad \text{(II. 39)}$$

In this reformulation of the quantum problem, the electron density and only the electron density, plays the key role.

As the external potential is easily known, if we knew the exact $F_{HK}[\rho]$, the previous equations would give the exact electron density. $F_{HK}[\rho]$ is defined independently of the external potential; this means that $F_{HK}[\rho]$ is a universal functional of $\rho(r)$.

III. The Kohn-Sham Equations

The problem is that among all the terms of $F_{HK}[\rho]$, only $J[\rho]$ is known while the explicit forms of the other two contributions ($T[\rho]$ and the non classical part of $V_{ee}[\rho]$) remain a mystery.

To understand how Kohn and Sham tackled this problem, it is convenient to begin with the exact formula for the ground-state kinetic energy:

$$T = \sum_{i}^{N} n_i \left\langle \chi_i \left| -\frac{1}{2}\nabla_i^2 \right| \chi_i \right\rangle \qquad \text{(II. 40)}$$

χ_i and n_i are the natural spin orbitals and their occupation number, respectively. The kinetic energy is a functional of the total electron density:

$$\rho(r) = \sum_{i}^{N} n_i \sum_{s} |\chi_i(x_i)|^2 \qquad \text{(II. 41)}$$

For an interacting system of interest, there are an infinite number of terms of the previous two equations. Kohn and Sham (1965) built a theory using simpler formula:

$$T_s = \sum_{i}^{N} \left\langle \chi_i \left| -\frac{1}{2}\nabla^2 \right| \chi_i \right\rangle \qquad \text{(II. 42)}$$

$$\rho(r) = \sum_{i}^{N} |\chi_i(x_i)|^2 \qquad \text{(II. 43)}$$

This is the special case having n_i=1 for N orbitals and n_i=0 for the rest; this representation of the kinetic energy and density describes N non interacting electrons. In this formalism, the interacting system is solved using a non-interacting system having the same density. The correction due the fact that the system is actually an interacting system is weak. Two major advantages exist with this solution. First, the wave function of a non interacting system can be represented as a Slater determinant of a single-electron orbital. Second the kinetic energy is not approximated (or at least the approximation is very good).

Kohn and Sham gave a Hamiltonian for a non-interacting system, having the same electron density and in which an effective local potential $v_s(r)$ is introduced:

$$\hat{H}_s = -\frac{1}{2}\sum_i^N \nabla_i^2 + \sum_i^N v_s(r) \qquad \text{(II. 44)}$$

This Hamiltonian operator does not contain any electron-electron repulsion terms since we are dealing with a non-interacting system of electrons. The ground state electron density is exactly ρ. The ground state wave function is represented by a Slater determinant formed with single-electron orbitals:

$$\Psi_0 = \frac{1}{\sqrt{N!}}\det[\chi_1(x_1)\chi_2(x_2)\ldots.\chi_N(x_N)] \qquad \text{(II. 45)}$$

This determinant is different from the Slater determinant defined in the HF methods since in this case φ_i^{KS} are the Kohn-Sham orbitals (or KS orbital). It can be found in a single-electron Schrödinger-type equation:

$$\hat{f}^{KS}\varphi_i^{KS} = \varepsilon_i\varphi_i^{KS} \qquad \text{(II. 46)}$$

where $\hat{f}^{ks}$ is the one-electron Kohn-Sham operator:

$$\hat{f}^{KS} = -\frac{1}{2}\nabla^2 + v_s(r) \qquad \text{(II. 47)}$$

At this stage the kinetic energy of the non-interacting system $T_S[\rho]$ has been introduced in the equations, and not the exact kinetic energy functional $T[\rho]$. The very clever idea of Kohn and Sham was to give the exact kinetic energy of the non-interacting reference system with the same density as the real interacting one (equation II. 48). Kohn and Sham then included the correction to the kinetic energy in the exchange-correlation term:

$$F[\rho] = T_S[\rho] + J[\rho] + E_{XC}[\rho] \qquad \text{(II. 48)}$$

where $T_S[\rho]$ is the kinetic energy of the non-interacting system, $J[\rho]$ is the classical electron-electron electrostatic interaction and E_{XC} is the exchange-correlation energy:

$$E_{XC}(\rho) = (T[\rho] - T_S[\rho]) + V_{ee}(\rho) - J[\rho] \qquad \text{(II. 49)}$$

$T[\rho]$ is the exact kinetic energy. In other words, the exchange-correlation energy E_{XC} is the functional which contains everything that is unknown. In spite of its name, E_{XC} contains not only the non-classical effects (i.e., self-interaction, exchange and correlation), which are the contributions to the potential energy, but also a portion belonging to the kinetic energy.

The total energy of an interacting real system is given by:

$$E[\rho(r)] = T_S[\rho] + J[\rho] + E_{XC}[\rho] + E_{Ne}[\rho] \qquad \text{(II. 50)}$$

$$= T_S[\rho] + \frac{1}{2}\iint \frac{\rho(r_1)\rho(r_2)}{r_{12}} dr_1 dr_2 + E_{XC}[\rho] + \int V_{Ne}\rho(r) dr$$

$$= -\frac{1}{2}\sum_i^N \langle \varphi_i^{KS} | \nabla^2 | \varphi_i^{KS} \rangle + \frac{1}{2}\sum_i^N \sum_j^N \iint |\varphi_i^{KS}(r_1)|^2 \frac{1}{r_{12}} |\varphi_j^{KS}(r_2)|^2 dr_1 dr_2$$

$$+ E_{XC}[\rho] - \sum_i^N \sum_A^{N_A} \int \frac{Z_A}{r_{1A}} |\varphi_j^{KS}(r_2)|^2 dr_1$$

The only term which is unknown is the exchange correlation energy E_{XC}, for which no explicit form can be given.

By applying the variational principle method to minimize this energy expression, we obtain the equation:

$$\left(-\frac{1}{2}\nabla^2 + \left[\int \frac{\rho(r_2)}{r_{12}} dr_2 + v_{xc}(r_1) - \sum_A^{N_A} \frac{Z_A}{r_{1A}}\right]\right)\varphi_i^{KS} \qquad \text{(II. 51)}$$
$$= \left(-\frac{1}{2}\nabla^2 + v_{eff}(r_1)\right)\varphi_i^{KS} = \varepsilon_i \varphi_i^{KS}$$

where v_{eff} is the effective Kohn-Sham potential, defined as follow:

$$v_{eff}(r) = \int \frac{\rho(r)}{r_{12}} dr + v_{XC}(r) + v(r) \qquad \text{(II. 52)}$$

where v_{XC} is the potential due to the exchange-correlation energy E_{XC}. v_{XC} is simply defined as the functional derivative of E_{XC} with respect to ρ:

$$v_{XC} = \frac{\delta E_{XC}}{\delta \rho} \qquad \text{(II. 53)}$$

The equations (II.52-II.54) form the famous Kohn-Sham (KS) equations. The major improvement (or at least what can distinguish KS from HF) is that KS fully incorporates the exchange-correlation effect of electrons, directly within the first steps of the formalism. The quality of KS equations can then just be improved following the improvements of the $E_{XC}[\rho]$ functional. The computational cost of solving Kohn and Sham equations scales formally as N^3 (where N is the number of Kohn-Sham orbitals), while for the post-HF methods it ranges from N^4 to N^7.

IV. Exchange-Correlation Functionals

The generation of approximations for E_{xc} has lead to a large expanding field of research; therefore we can find many different functionals which are more or less appropriate for a particular study. One of the most well-known problems encountered by the DFT methods is the weak quality of the description of long-range interactions. Recent functionals have introduced dispersive terms (DFT-D). An accurate description of the activation barriers (and thus kinetics) strongly depends on both the molecular system and the functional. For such studies a systematic methodological investigation is required.

IV.1. Local Density Approximation

The LDA approach is based on the jellium notion (i.e., uniform gas of electrons "moving" on a "matrix" constituted of positive charges, which forms a positive background distribution). This approach allows to write E_{XC} as a function of the exchange-correlation energy per electron of a uniform gas of density ρ:

$$E_{XC}^{LDA}[\rho] \approx \int \rho(r)\varepsilon_{xc}(\rho(r))dr \qquad \text{(II. 54)}$$

That is, E_{XC} is expressed as a function of local physical properties and is so-called the local density approximation (LDA). $\varepsilon_{xc}(\rho)$ can be split into the both exchange and correlation contributions:

$$\varepsilon_{xc}(\rho) = \varepsilon_x(\rho) + \varepsilon_c(\rho) \qquad \text{(II. 55)}$$

The exchange part (i.e., the exchange energy of a uniform gas of electrons of a particular density) can be obtained by the Slater exchange expression from the Hartree-Fock approximation. There is no such explicit expression of the correlation part. Based on numerical quantum Monte Carlo simulation of a homogeneous electron gas, Vosko, Wilk and Nusair (VWN) have proposed different expressions, among them SVWN is the most widely used [13]. When the electronic density is decomposed into both the contributions of spin up and spin down (equation II. 56), the exchange-correlation functional can also be developed in an unrestricted scheme (equation II. 57). This approach is called Local Spin Density Approximation (LSDA).

$$\rho(r) = \rho_\alpha(r) + \rho_\beta(r) \qquad \text{(II. 56)}$$

$$E_{XC}^{LSD}[\rho_\alpha, \rho_\beta] \approx \int \rho(r)\varepsilon_{xc}(\rho_\alpha(r), \rho_\beta(r))dr \qquad \text{(II. 57)}$$

LDA is based on approximations that gave good results for solid physic, since the jellium is a good description of electron conduction in metals and often in semi-conductors. However this is usually not sufficient for the chemistry in solution.

IV.2. The Generalized Gradient Approximation

A natural (for mathematicians) progression beyond the local density approximation is the gradient expansion approximation (GEA), in which E_{XC} exchange-correlation is written as a Taylor expansion (LDA is the first order of GEA):

$$E_{XC}^{GEA}[\rho] \approx \int \rho\,(r)\varepsilon_{xc}(\rho)dr + \sum C_{XC}\frac{\nabla\rho}{\rho^{4/3}} + \cdots \qquad \text{(II. 58)}$$

This method did not give the expected improvement. This comes from the fact that such a mathematical treatment makes that the correction (from a non-interactive to an interactive system) lost its physical meaningful. The generalized gradient approximation (GGA) solves this problem. In this GGA functionals the density gradient is included to ensure the validity of the different conditions that give meaningful. As a consequence GGA functionals are strongly parameterized.

The typical form for a GGA functional is given by:

$$E_{XC}^{GGA} \approx \int \rho(r)\,\varepsilon_{xc}(\rho,\nabla\rho)dr \quad \text{(II. 59)}$$

In the GGA approximation, the exchange and correlation contributions can be treated separately:

$$E_{XC}^{GGA} = E_{X}^{GGA} + E_{C}^{GGA} = \int f(\rho_\alpha,\rho_\beta,\nabla\rho_\alpha,\nabla\rho_\beta)\,dr + E_{C}^{GGA} \qquad \text{(II. 60)}$$

The exchange functional is given by:

$$E_{X}^{GGA} = E_{X}^{LDA} - \int F[s(r)]\rho^{4/3}(r)dr \quad \text{(II. 61)}$$

where s is a dimensionless parameter, so-called the density gradient:

$$s(r) = \frac{|\nabla\rho(r)|}{\rho^{4/3}(r)} \qquad \text{(II. 62)}$$

Two types of GGA exchange functionals exist. In the first type, the functionals fitted on the exchange energy of rare gas (e.g., B, CAM, FT97, O, PW, mPW). For the second type, the functionals are mathematically developed and based on rational function expansions (e.g., P, P86, LG, P, PBE, mPBE). In this case, no empirically optimized parameters are included.

Besides, correlation functionals are empirically built, which gives relatively complicated mathematical expressions (e.g., LYP, P86, PW91).

The exchange-correlation GGA functionals are the combinations of exchange and correlation terms (e.g., BLYP, OLYP, BP86) [14-18]

IV.3. Meta-GGA Functionals

Meta-GGA (MGGA) functionals depend explicitly on the Laplacian $\Delta\rho$ and on the local kinetic energy density [19, 20]. The typical form of MGGA functionals is given by:

$$E_{XC}^{MGGA} = \int \rho(r)\, \varepsilon_{xc}(\rho, |\nabla\rho|, \Delta\rho, \tau) dr \quad \text{(II. 63)}$$

where the kinetic energy density τ is given by:

$$\tau = \frac{1}{2}\sum_{i} \left|\nabla\varphi_i^{KS}\right|^2 \qquad \text{(II. 64)}$$

IV.4. Hybrid Exchange Functionals

The idea of building hybrid functionals came from the observation that the exchange functional can exactly been obtained within the HF theory. Thus authors proposed to replace E_X^{LDA} or E_X^{GGA} by the exact HF exchange expression, except that KS orbitals were used in place of HF orbitals. The approximated mathematical expression will be used only for correlation.

$$E_{XC} = E_X^{HF} + E_C^{KS} \quad \text{(II. 65)}$$

Nonetheless the results obtained were not as good as expected compared to pure GGA. A good compromise was to include just a percentage of the exact HF exchange and to mix with DFT exchange.

$$E_{XC} = E_X^{HF} + z(E_{XC}^{DFT} - E_X^{HF}) \quad \text{(II. 66)}$$

Or

$$E_{XC} = (1 - a)E_{XC}^{DFT} + aE_X^{HF} \quad \text{(II. 67)}$$

If a=0.5, the hybrid functional is the so-called "Half-and-Half (HandH)"

The exchange-correlation in B3LYP has been defined with three parameters as follows:

$$E_{XC}^{B3LYP} = (1 - a)E_X^{LSDA} + aE_X^{HF} + bE_X^B + E_C^{LSDA} + cE_C^{LYP} \quad \text{(II. 68)}$$

Over the past decades DFT has appeared as an efficient tool to compute the electronic properties (electronic energy, bond dissociation enthalpy, ionization potential…) of various molecular systems. However, the reliability of DFT is strongly functional-dependent.

IV.5. Recent developments

It is well known that DFT had two main limits: (i) the self-interaction error and (ii) the bad description of long-range interactions. The community of theoretical chemists have developed a plethora of new functionals to correct them.

IV.5.1. Self-interaction Error

For a one-electron system described by the Hartree-Fock formalism, the Coulomb term (J_{ij}) is cancelled by the exchange term (K_{ij}). However trivial DFT functionals described an artificial interaction term of one electron with itself. Many functionals correct it using the Self-interaction Correction (SIC) developed by Perdew and Zunger [21]. SIC is basically based on the fact that "exchange-correlation energy of a single, fully orbital must exactly cancel its self-direct Coulomb energy". In this purpose it is possible to include a term in the exchange-correlation functional in order to have $E_{XC}[\rho] = -J[\rho]$ in one-electron systems.

IV.5.2. Long-range interactions

DFT does not include dispersive contribution in r^{-6}, which is useful to describe long H-bond, π-stacking, etc... Possible solutions are being pursued. (i) An empirical-based potential in r^{-6} can be included in the exchange-correlation functional [22] (Dispersive-corrected

density functional DFT-D, e.g., B3LYP-D, PBEPBE-D). (ii) The long-range interactions can be described as a second-order perturbation of exchange-correlation functional (e.g., B2-PLYP [23]. (iii) It is also possible to separate short-range interactions and long-range interactions using specific function (Range-separated hybrid functional, e.g., CAM-B3LYP).

IV.5.3. New Hybrid-MetaGGA DFT (HMDFT) functionals

The above-mentioned problem of DFT validation was a major activity domain in the DFT community. Some groups developed new functionals able to describe many different properties (e.g., thermodynamics, kinetics, non-covalent interactions). The combination of hybrid functionals and M-GGA in exchange and correlation functionals, respectively was a good approach to evaluate accurately various chemical properties. For example, Zhao and Truhlar recently developed MPWB1K and MPW1B95, which calculate kinetics and thermodynamics at the same time with a better accuracy than with trivial functionals [24].

Electronic Spectroscopy

I. Electronic transition and selection rules

The absorption of an electromagnetic radiation (e.g., UV/visible light) by a molecular system induces an electronic transition from the ground state (GS) to an excited state (ES).

The excited electron "jumps" from the potential energy surface of GS (S_0) to that of ES (S_1) (Fig. II.1). Starting from the GS optimal geometry, the molecular system reaches the potential energy surface of the targeted ES. The light absorption is usually a very fast physical process which does not allow geometrical re-organization. The subsequent electronic transition is so-called the vertical transition or Franck-Condon transition E_{vert} (abs) (Fig. II.1). On the timescale, the geometry of ES ultimately relaxes to its own minimum (the optimized structure of ES). The difference between the adiabatic and vertical transition energies is called reorganization (or equilibration) energy ΔE_{eq} (Fig. II.1), this can be directly correlated with the bandwidth of the electronic transition as observed in the experimental spectrum. In the latter, the adiabatic transition can be identified (in a first approximation) with the onset of the absorption, while the vertical transition can be identified (again in a first approximation) with the absorption maximum [25]. The reverse case (emission) followed the same process. Note that excitation and emission energies (ΔE) are often expressed in terms of the corresponding wavelength λ:

$$\Delta E = \frac{hc}{\lambda} \qquad (II.69)$$

where h is the Planck's constant, c the speed of light in vacuum, and λ the radiation wavelength (the larger ΔE, the shorter λ). The difference between the vertical excitation and emission energies (or wavelengths) is called the Stokes shift.

Within the orbital scheme, electronic transitions occur from occupied to non-occupied (virtual) molecular orbitals. In theory the number of electronic transitions is huge. They may occur between frontier orbitals i.e., from HOMO (highest occupied molecular orbital), HOMO-1, HOMO-2… to LUMO (lowest unoccupied molecular orbital), LUMO+1, LUMO+2… (e.g., catechol in Fig. II.2). Nonetheless all the electronic transitions are not allowed, according to the selection rules, which are highly related to molecular symmetry.

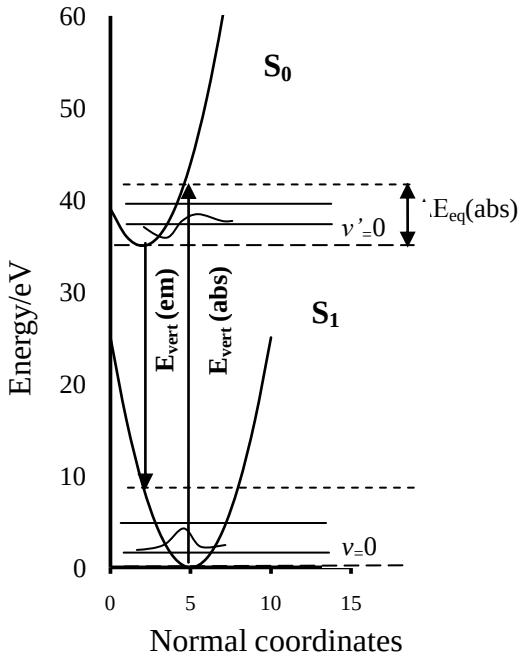

Figure II.1: Schematic representation of the vertical electronic transitions between GS (S_0) and ES (S_1), showing E_{vert} and ΔE_{eq}.

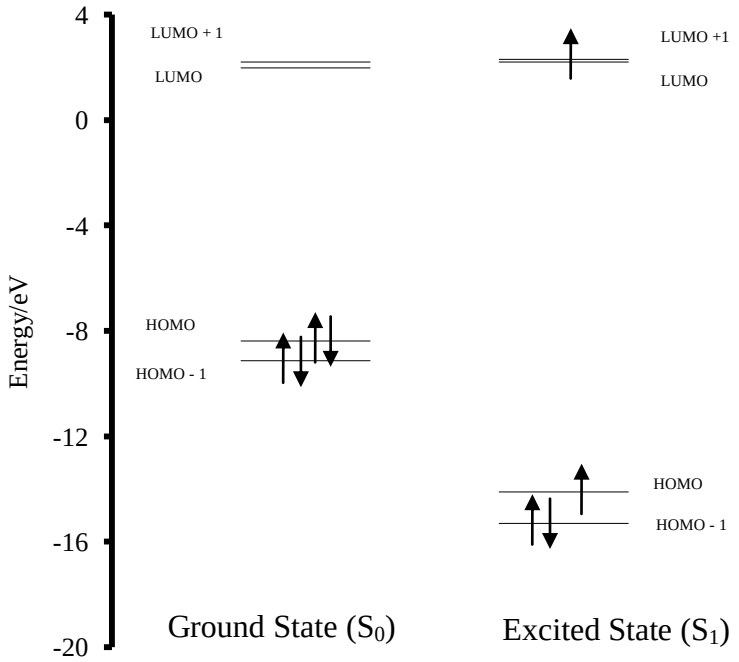

Figure II.2: Electronic transition between frontier orbitals of catechol (RCIS/6-311+G(d,p), this is the case of one-electron transition (from HOMO of S_0 to the LUMO of S_1)

$$^1\Psi_1 = \left|\psi_1^2\psi_2^2\psi_3^2 \ldots \psi_{N/2}\overline{\psi_a}\right\rangle \quad \text{(II. 72)} \qquad ^3\Psi_1 = \left|\psi_1^2\psi_2^2\psi_3^2 \ldots \psi_{N/2}\psi_a\right\rangle \quad \text{(II. 73)}$$

In order to understand and to predict these rules the wave function may be considered as a time-dependent wave function Ψ since an optical excitation (e.g., UV/visible light) is an oscillating electric field given by:

$$F = e\varepsilon \quad \text{(II. 74)}$$

where the oscillating electric field can be written:

$$\varepsilon = \varepsilon_0 \cos 2\pi\nu t = \frac{1}{2}\varepsilon_0\left(e^{i2\pi\nu t} + e^{-i2\pi\nu t}\right) \quad \text{(II. 75)}$$

The global wave function of an excited system can be written as a linear combination of the wave function of both GS and ES (Ψ_0 and Ψ_1, respectively):

$$\Psi(r,t) = c_0\Psi_0(r,t) + c_1\Psi_1(r,t) \quad \text{(II. 76)}$$

At time t = 0 (no optical excitation) $c_0 = 1$ and $c_1 = 0$. At time t (during the excitation) c_1 varies, and this is what we are dealing with. The gradient of c_1 *vs* time gives the "filling" of the corresponding ES. If $\frac{dc_1}{dt}$ is high, the transition is allowed. If it is zero, the transition is forbidden.

A classical mathematical treatment allows to obtain:

$$\frac{dc_1}{dt} = \frac{\varepsilon_0}{2i\hbar}\left(e^{i(E_1 - E_0 + h\nu)t/\hbar} + e^{i(E_1 - E_0 - h\nu)t/\hbar}\right)\int_{-\infty}^{+\infty}(e.r)\Psi_1^*\Psi_0\,dr \quad \text{(II. 77)}$$

where E_0 and E_1 are the energies of GS and ES, respectively.

The integral term is the so-called transition dipole moment and is denoted M_{01}. This parameter is at the very heart of the electronic transition science. If it is different from zero it means that the transition is allowed while if it is zero the transition is forbidden. To be more precise, what is exactly required is the probability of the transition i.e., the probability of being in the excited state $c_1^* c_1$. This probability can be obtained for a light having different wave lengths (e.g., white light). To take into account all the possible excitation frequencies, $c_1^* c_1$ is calculated as the integral over ν $(0 \rightarrow \infty)$:

$$c_1^* c_1 = \frac{\varepsilon_0^2 M_{01}^2 t}{4\hbar^2} \qquad \text{(II. 78)}$$

or

$$\frac{d(c_1^* c_1)}{dt} \propto \varepsilon_0^2 M_{01}^2 \qquad \text{(II. 79)}$$

This can be extrapolated to any $ES_n \rightarrow ES_{m>n}$ transition, which is ruled by the transition dipole moment M_{nm}. In order to know if an optical transition is allowed (i.e., a specific GS → ES_n or $ES_n \rightarrow ES_{n+m}$) one needs to estimate the transition dipole moment, to draw up the selection rules. A first physico-chemical interpretation of M_{nm} can be given regarding in details its mathematical expression. First of all, it can be noted that e.r (eq. II.77) is an electric dipole. Thus, the transition dipole moment is, by definition, the dipole moment associated to the electronic transition. It corresponds to the electronic changing (change in the electronic distribution) during the electronic transition. Then to be non zero, both electronic states must be relatively similar. To be more precise and from a symmetrical point of view, M_{01} is non zero if the product contains the totally symmetrical element.

It must be stressed that this is the ideal case, since here we assume that a transition occurs between only two states. Actually, the Nature often allows a mixture of different « single » transitions.

So the transition moment integral can be re-written as follows:

$$M_{01} = \int \Psi_1^* \hat{\mu} \Psi_0 d\tau \qquad \text{(II. 80)}$$

where $\hat{\mu}$ is the dipole moment operator. It can be divided into two contributions, one from the nucleus $\hat{\mu}_n$ and the other from electrons coordinates $\hat{\mu}_e$:

$$\hat{\mu} = \hat{\mu}_n + \hat{\mu}_e \qquad \text{(II. 81)}$$

The wave function can also be written as the product of three contributions:

$$\Psi = \Psi_{es}\Psi_v = \Psi_e \Psi_s \Psi_v \qquad \text{(II. 82)}$$

where Ψ_e, Ψ_s and Ψ_v are the electronic, spin and nuclear (vibrational) wave functions, respectively. The dipole moment can thus be written:

$$M_{01} = \int \Psi_{1es}^* \Psi_{1v}^* (\hat{\mu}_n + \hat{\mu}_e) \Psi_{es} \Psi_v d\tau \qquad \text{(II. 83)}$$

$$= \int \Psi_{1es}^{*}\, \Psi_{1v}^{*} \hat{\mu}_n \Psi_v d\tau + \int \Psi_{1es}^{*}\, \Psi_{1v}^{*} \hat{\mu}_e \Psi_{es} \Psi_v d\tau$$

$$= \int \Psi_{1es}^{*}\, \Psi_{es} d\tau_{es} \int \Psi_{1v}^{*} \hat{\mu}_n \Psi_v d\tau_n + \int \Psi_{1v}^{*}\, \Psi_v d\tau_n \int \Psi_{1es}^{*}\, \hat{\mu}_e \Psi_{es} d\tau_{es}$$

As the spin-orbitals χ_{es} are orthogonal, the first integral is zero, therefore the dipole moment have the form:

$$M_{01} = \int \Psi_{1v}^{*}\, \Psi_v d\tau_n \int \Psi_{1e}^{*}\, \hat{\mu}_e \Psi_e d\tau_e \int \Psi_{1s}^{*}\, \Psi_s d\tau_s \quad \text{(II. 84)}$$

The first term $\int \Psi_{1v}^{*}\, \Psi_v d\tau_n$ (called the Franck-Condon factor) is not necessarily zero since the two vibrational states may belong to two different electronic states and therefore are not necessarily orthogonal.

The second term gives the orbital selection rules; if $\int \Psi_{1e}^{*}\, \hat{\mu}_e \Psi_e d\tau_e$ is nonzero, the electronic transition is orbitally allowed. This is strongly related to the symmetry of the system since an electronic transition is orbitally-allowed if and only if the product of the irreductible representations of both orbitals and the dipole ($\Gamma(\Psi_{1e}) * \Gamma(\hat{\mu}_e) * \Gamma(\Psi_e)$) contains the totally symmetric irreductible representation of the point group of the molecule.

The third term gives the spin selection rules. It is nonzero (allowed transition) if and only if the multiplicities of the two states involved in the transitions are identical (i.e., singlet→singlet; triplet→triplet).

If an electronic transition is spin and orbitally-allowed, it is called fully allowed and their intensity can be intense. The Franck-Condon integral only modulates the intensity of the transition, which is essentially influenced by the electronic transition (spin and orbital selection rules). It corresponds to the overlap between the vibrational wave functions of GS and ES involved in the vertical transition.

If we want to consider the hyperfine structure of an absorption spectrum, we have to explicitly consider the different vibrational states (Fig. II.1). The selection rules are then given by combining the first two terms and check if the corresponding integral equals zero or not:

$$M_{01} = \int \Psi_{1e}^{*} \Psi_{1v}^{*}\, \hat{\mu}_e \Psi_e \Psi_v d\tau_{en} \int \Psi_{1s}^{*}\, \Psi_s d\tau_s \quad \text{(II. 85)}$$

More practically an allowed transition will produce an absorption band more or less intense. The intensity is measured by the extinction coefficient ε as defined by the Beer Lamber law:

$$A = \varepsilon CL \qquad (II.\ 86)$$

where A is the measured absorbance, C is the concentration of the molecule in the solution and L is the pathway crossed by the light. When integrating over ν, another characteristic parameter can be defined:

$$f = 4.32 * 10^{-9} \int_0^{\infty} \varepsilon(\bar{\nu}) d\bar{\nu} \quad (II.\ 87)$$

where f is the dimensionless oscillator strength (the higher f, the larger the transition dipole moment). The integral represents the area under the absorption band given in molar coefficients ε (in $M^{-1}cm^{-1}$), when plotted *vs* ν (in cm^{-1}).

II. Excited states and Time-Dependent Density Functional Theory

II.1. Time-Dependent DFT

While the description of the potential energy surface of GS can be achieved by classical quantum methods (HF, DFT, semi-empirical), the potential energy surface of ES is usually badly reproduced. This is still a challenge of modern quantum chemistry to give an accurate description of ES. Different methods were developed to accurately deal with ES (e.g., time-dependent HF and time-dependent configuration interaction). Most of the methods solved the problem within the perturbation theory, in which the perturbation is a time-dependent excitation (e.g., an oscillating electric field). The time-dependent DFT (TD-DFT) is relatively recent. It has been developed after the publication of Runge and Gross who gave a generalized Hohenberg-Kohn-Sham formalism to time-dependent systems [26]. TD-DFT is the natural extension of DFT and is the "excited-state density functional theory". Most of the developments and uses are achieved within a linear-response theory, which is enough to remarkably reproduce the behaviour of most of the physical systems (e.g., UV/visible absorption).

Runge and Cross first established the extension of the Hohenberg and Kohn theorems. They proved that for two different external potentials up to a time-dependent constant $v_1(t) \neq v_1(t) + c(t)$ the corresponding time-dependent densities $\rho_1(r,t)$ and $\rho_2(r,t)$ (evolving from a common initial state $\Psi_0 = \Psi(t_0)$) are different. In other words, $\rho(r,t)$ uniquely determines $v_{ext}(r,t)$ (up to a time-dependent constant). The wave function is in turn determined. Similarly to the Hohenberg-Kohn theorem, the Runge-Gross theorem also states that as soon as $\rho(r,t)$ is defined, all observables can be obtained.

To continue the extension of the Hohenberg-Kohn-Sham formalism to the corresponding time-dependent theory, one can rewrite the many-electron problem using a time-dependent KS non-interacting system, having the same density than the fully interacting system:

$$\rho(r,t) = \sum_{i}^{occ} \left|\varphi_i^{KS}(r,t)\right|^2 \qquad \text{(II. 88)}$$

The time-dependent KS equations are:

$$i\frac{\partial}{\partial t}\varphi_i^{KS}(r,t) = \left[-\frac{\nabla^2}{2} + v_{KS}(r,t)\right]\varphi_i^{KS}(r,t) \quad \text{(II. 89)}$$

where

$$v_{KS}(r,t) = v_{ext}(r,t) + v_{hartree}(r,t) + v_{XC}(r,t) \qquad \text{(II. 90)}$$

in which $v_{ext}(r,t)$ is the external potential, $v_{hartree}(r,t)$ is the classical electrostatic interaction between the electrons and $v_{XC}(r,t)$ is the exchange-correlation potential, which contains all the electron-electron interactions (including the non-classical interactions) and the correction to the exact kinetic energy of the interacting system.

If we knew the exact Kohn-Sham potential v_{KS}, we would obtain the exact Kohn-Sham orbitals and therefore the correct electron density of the system. However as for the time-independent problem, v_{KS} is not known exactly and must be approximated. It can be written as follows:

$$v_{XC}[\rho](r,t) = \frac{\partial E_{XC}[\rho]}{\partial \rho} \qquad \text{(II . 91)}$$

Actually in the time-dependent physical problem, an "action functional" is defined in place of the energy.

$$\mathcal{A}[\psi] = \int_{t_0}^{t_1} \left\langle \Psi(t)\left| i\frac{\partial}{\partial t} - \hat{H}(t)\right|\Psi(t)\right\rangle dt \quad \text{(II. 92)}$$

and the exchange correlation potential is written as follows:

$$v_{XC}(r,t) = \left.\frac{\partial A_{XC}}{\partial \rho(r,t)}\right|_{\rho(r,t)} \qquad \text{(II. 93)}$$

When the molecular system absorbs light, the external potential is not only the interaction between electrons and nuclei but also the physical perturbation (i.e., an oscillating electric field $\varepsilon = \varepsilon_0 \cos 2\pi\nu t = \varepsilon_0 \cos\omega t$). If the perturbation can be considered weak as compared to

the global energy of the system, it can be treated within the linear response theory i.e., the external potential is:

$$v_{ext}(r,t) = v_0(r) + \varepsilon_0 \cos 2\pi\nu t \qquad \text{(II. 94)}$$

where $v_0(r)$ is the classical electron-nuclei attraction potential given by:

$$v_0(r) = -\sum \frac{Z_A}{R_A - r} \qquad \text{(II. 95)}$$

The derived equations form must be solved by a self-consistent procedure.

Summary:

$$\rho(r,t) = \sum_{i=1}^{N} \left|\varphi_i^{KS}(r,t)\right|^2 \qquad \text{(II. 96)}$$

$$v^{KS}(r,t) = v(r,t) + \int dr' \frac{\rho(r',t)}{|r - r'|} + \frac{\delta A_{XC}[\rho]}{\delta\rho(r,t)} \qquad \text{(II. 97)}$$

$$i\frac{\partial}{\partial t}\varphi_i^{KS}(r,t) = H^{KS}(r,t)\varphi_i^{KS}(r,t) \qquad \text{(II. 98)}$$

The initial electron density $\rho_0(r)$ can be self-consistently obtained from the time-independent DFT calculation.

II.2. Polarizable continuum model in time-dependent DFT

Most of the UV/visible spectra performed for natural compounds are achieved in solution. The theoretical evaluation of the excited states should systematically be performed including solvent effects.

The use of explicit solvent models is unfeasible to study the optical properties of series of natural compounds. Only implicit models can be used to achieve such calculations. Among these types of models the polarizable continuum models (PCM) have extensively been studied and allowed an accurate estimation of most properties for stationary points.

PCM models consider that the studied structure(s) is confined in a shape-adapted cavity surrounded by a dielectric continuum characterized by its dielectric constant (ε = 78.4 for water). The electostatics of the solute-solvent system is ruled by the Poisson equation:

$$\mathrm{div}[\epsilon(r)\nabla V(r)] = -4\pi\rho(r) \qquad (II.99)$$

in which ρ is the sum of the electronic and nuclear charge densities of the solute and V(r) is the total electrostatic potential. When writing the boundary conditions at the cavity surface, an apparent charge σ' can be defined on the surface. This takes the polarization of the solute (which in turn polarizes the solvent according) into account. The global solute-solvent interaction is the reaction electrostatic potential. The charge σ' is connected to this potential V by the response operator Q defined by the electrostatic theory [27-29].

$$\sigma' = Q.V \qquad (II.100)$$

To solve this problem it is more convenient not to consider a discrete instead of a continuous charge distribution on the surface. The cavity surface is divided in small elements s (tesserae), each of them associated to a point charge. The resolution becomes a matrix problem.

$$\sigma'(s) = Q(s,s').V(s) \qquad (II.101)$$

The solute-solvent interaction is given by:

$$E_{int} = \int_{\Gamma}\int_{\Gamma} V(s)Q(s,s')\,V(s')dsds', \quad \Gamma \text{ being the cavity surface} \qquad (II.102)$$

The PCM response can then be inserted in the SCF procedure. Both the free energy and the solute electronic distribution are then affected according to the solute-solvent interaction until convergence of the wave function is reached. The PCM-type contribution is introduced in the Fock (or KS operator) as a pertubative effect:

$$F = F_0 + v^{PCM} \qquad (II.103)$$

where $v^{PCM}(r) = \frac{\partial G_{int}}{\partial \rho^{el}(r)}$

Within the TD-DFT formalism the external potential varies in time and the time-dependent KS-equation is considered (eq. II.97 and 98, respectively). The solvent contribution is then added as a perturbation of the time-dependent operator, as for the time-independent problem. As a first approximation the time-independent external potential can be used, with the instantaneous value of the density. Most time-dependent properties can thus be evaluated by using the density variation instead of the electronic density. All the different terms depending on the electronic density are re-written with the density variation e.g., for equation (II.102) that describes the PCM response.

$$v^{PCM}[\delta\rho^{el}](r) = \int_\Gamma \int_\Gamma \delta V(s',\omega) Q(\epsilon_{opt}; s', s) \frac{1}{|s-r|} ds ds' \qquad (II.104)$$

$$\delta V(s',\omega) = \int_{R^3} \frac{\delta\rho^{el}(r',\omega)}{|s'-r'|} dr' \qquad (II.105)$$

This reflects the variation of the solute-solvent interaction due to a sudden change in the solute electronic density, as a consequence of the variation of the external potential. As for the time-independent problem, this is included in the SCF procedure. Different mathematical refinements of such a procedure have been implemented in the Gaussian software, depending on the version.

As far as a proper basis set is used (triple-dzeta basis set + polarisation and diffuse functions), TD-DFT/PCM calculations can provide a very good agreement with the observed excitation energies [30-32]. However we believe that this is highly system-dependent and benchmarks are often achieved for relatively small systems. As a first methodological stage for polyphenols, TD-DFT/PCM values of λ_{MAX} are compared to experimental values obtained in methanol (Chapter 4). To definitely confirm that TD-DFT/PCM is accurate to evaluate UV/visible spectra for polyphenols, a systematic investigation should be performed, comparing wavelength experimentally obtained both in the gas phase and in different solvents.

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Chapter 3: Structure activity relationship for the free radical scavenging capacity of polyphenols

H-atom acceptor capacity of free radicals used in antioxidant measurements (e.g., DPPH and peroxyradicals ROO$^{\bullet}$ and LOO$^{\bullet}$)

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Important note for the reader: This article has been published in International Journal of Quantum Chemistry. I only managed the part concerning DPPH, but to keep consistency the entire manuscript is incorporated in this PhD with the kind agreement of the other co-authors.

Free radical scavenging properties of Guaiacol oligomers: A combined experimental and quantum study of the guaiacyl-moiety role

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A density functional theory DFT study of polyphenols compounds: Quantitative structure antioxidant activity relationship

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Chapter 4: UV/visible absorption by polyphenols

Colors of natural polyphenols: a TD-DFT study

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Effect of oligomerisation on UV absorption of π-conjugated (guaiacol)n=1-4:

comparison between experience and TDDFT

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Conclusion and perspectives

The important points of the present work can be summarized as follow. First the antioxidant properties of polyphenols have been better rationalized. We established why a compound is active; or in other words, which chemical characteristics enhance the activity? E.g., π-electron conjugation plays a crucial role to stabilize the phenoxy radical formed after HAT. A second HAT has been demonstrated to strongly enhance the activity, as well. We have clearly established the role of the guaicyl moiety, which is largely found in many natural and hemi-synthetic polyphenols. BDE of the phenolic OH groups appear as the major descriptor of the antioxidant activity. The number of OH groups (n_{OH}) and H-bonds are two dependent descriptors. The capacities of deprotonation and electron transfer are also two minor descriptors. We confirmed the role of BDEs but also of a new parameter: BDE_D, or similarly n_{OH}×BDE. DFT allows an accurate description of this biological activity and we are now able to accurately predict the activity of new compounds. This is crucial for pharmaceutic and cosmetic industries but also for academic groups having large collections of natural product. From this study we expect to establish a database incorporating many computed predictive descriptors. The use of quantum chemistry maybe extended to other biological activities.

Polyphenols are partly responsible for the variation of colour in flowers, fruit and vegetables. The relationship between the UV/visible spectra and the chemical structures are well-reproduced by TD-DFT. The results demonstrate that the excited states and the allowed electronic transitions depend on the number of OH groups (λ_{MAX} increased with n_{OH}), the presence or not of the 2,3-double bond in flavonoids, the presence or not of the 3-OH group in flavonoids, the number of units n along the chain, the solvent and the planarity. On the basis of this study, TD-DFT can be seen as a very effective tool to reproduce the capacity of natural polyphenols to absorb UV/visible light. This is of great importance to (i) predict shifts in the

UV/visible spectra of new compounds, (ii) explain plant and fruit colours, (iii) understand UV-induced isomerisation, (iv) predict their capacity to be used as sunscreen and (v) predict co-pigmentation due to the formation of polyphenol-complexes in plants (berries, tomatoes).

It must also be stressed as a general conclusion that hybrid functionals (e.g., B3P86) are particularly well-adapted to reproduce most of the properties of polyphenols. Its use must now be recommended in most of the investigations on these compounds, as for a researchers want to establish predictive activities and properties.

The main perspective of the present work is to extend the use of the quantum descriptors showed to be important for large series of compounds. This study was limited to fourty three and thirty two different polyphenols for the antioxidant activity and the UV/vis property, respectively. This is of academic importance and was an inescapable stage to validate the methodology and the robustness of the descriptors. Nonetheless the high quality of the correlation between the quantum descriptors (e.g., BDE and BDE_D) and the activity/property makes this work mature to be used at an "industrial" stage, or at least with academic having very large collections of compounds (more than 1000). The future idea is to automatise the calculations of these quantum descriptors for numerous members of numerous series of compounds. As it, databases could be built and systematically be used by research groups of e.g., cosmetology firms. Such databases would also be valuable to guide the synthetis of new polyphenols with optimized capacities to decrease oxidative stress in the human organism.

Annex

Supplementary Materials

Free radical scavenging properties of Guaiacol oligomers: A combined experimental and quantum study of the guaiacyl-moiety role.

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