

Dedicated to Professor Victor-Emanuel Sahini  
on the occasion of his 80th anniversary

## CORRELATIONS BETWEEN ELECTROCHEMICAL ACTIVITY OF PHENOTHIAZINE DERIVATIVES MODIFIED GRAPHITE ELECTRODES AND SOME STRUCTURAL AND MOLECULAR CHARACTERISTICS

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A comparative study on the correlation between the electrochemical parameters (formal standard potential, surface coverage, heterogeneous electron transfer rate constant, and deactivation rate constant) and the structural and molecular characteristics of four phenothiazine derivatives containing two linearly condensed phenothiazine moieties (16*H*,18*H*-dibenzo[*c,l*]-7,9-dithia-16,18-diazapentacene, DDDP; dibenzo[*c,l*]-16,18-diacetyl-7,9-dithia-16,18-diazapentacene, ADDP; dibenzo[*c,l*]-16,18-dibenzoyl-7,9-dithia-16,18-diazapentacene, BDDP; 16*H*,18*H*-dibenzo[*c,l*]-7,9-dithia-16,18-diazapentacene-7,7,9,9-bis-dioxide, DDPSO<sub>2</sub>) and four highly chlorinated phenothiazine derivatives (1,1,2,2,3,3,4,6,7,8,9-undecachloro-1,2-dihydro-phenothiazine, UDP; 1,2,3,4,6,7,8,9-octachloro-phenothiazinyl, OCP; 1,2,4,6,7,8,9-heptachloro-3-hydroxy-phenothiazine, HHP; 1,2,4,6,7,8,9-heptachloro-3*H*-phenothiazine-3-one, HPO), adsorbed on spectrographic graphite, is reported. In order to estimate their energy related parameters (total electronic energies, enthalpies of formation, and energies of frontier orbitals, HOMO and LUMO), electron distributions (charge densities and bond orders), and geometrical parameters (bond lengths, bond angles and molecular areas), semi-empirical SCF MO calculations (AM1 and PM3) for the optimized geometries of the investigated compounds and their ionized species were performed.

### INTRODUCTION

The phenothiazine derivatives present a special interest for obtaining modified electrodes having important practical applications in biomedical, environmental and biotechnological analyses.<sup>1</sup> Among these applications, the electrochemical oxidation of reduced  $\beta$ -nicotinamide adenine dinucleotide (NADH) has attracted much interest because of the large number of redox enzymes (400) that employ this coenzyme, resulting in many different electrochemical biosensors designed for the determination of various analytes of great importance in clinical and food chemistry.<sup>2-6</sup>

There are many studies concerning structure - properties correlations referring to charge transfer process kinetics using various MO methods, beginning with  $\pi$  electron methods, such as the

LCAO-Hückel (HMO)<sup>7</sup> and Pariser-Parr-Pople (PPP),<sup>8</sup> and continuing with all-valence electrons methods, such as extended Hückel (EH),<sup>9</sup> and the newer semi-empirical approximations AM1,<sup>10-12</sup> and PM3.<sup>13</sup> The influence of the solvent on the electronic structure was sometimes included, by the use of various simplified models.<sup>11-13</sup> The electrocatalytic oxidation process of NADH has also been investigated and correlations between the formal standard potential and the electrocatalytic rate constants for NADH oxidation were established.<sup>14</sup>

In this context, four new synthesized phenothiazine derivatives (DDDP compounds from Scheme 1) based on the 16*H*,18*H*-dibenzo[*c,l*]-7,9-dithia-16,18-diazapentacene structure<sup>15,16</sup> and four highly chlorinated phenothiazine derivatives (Ph-Cl compounds from Scheme 2)<sup>17,18</sup> were used to

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obtain modified graphite electrodes by simple adsorption.<sup>19-21</sup> Their electrochemical behavior and electrocatalytic activity towards NADH oxidation was investigated and it was observed that DDDP derivatives present the most convenient redox response, the highest values of heterogeneous electron transfer rate constant, while Ph-Cl derivatives present the smallest value of the formal standard potential at the optimum pH value for NADH electro-oxidation (pH 7).

Our investigation on the electrochemical behavior of these electrodes made possible the determination of their main electrochemical parameters such as: formal standard potential,  $E^0$ ; surface coverage,  $\Gamma$ ; heterogeneous electron transfer rate constant,  $k_s$  and deactivation rate constant,  $k_{\text{deact}}$ . In order to find out possible correlations between these experimental quantities and the structural characteristics of the organic molecules, semi-empirical SCF MO calculations were done by two methods (AM1 and PM3). These computations allowed the estimation of the geometrical parameters for the optimized molecular geometry of these compounds, as well as the polarizabilities, some energy related parameters and electron distributions. All these calculated parameters were correlated with the electrochemical characteristics experimentally found.

## EXPERIMENTAL AND COMPUTATIONAL PART

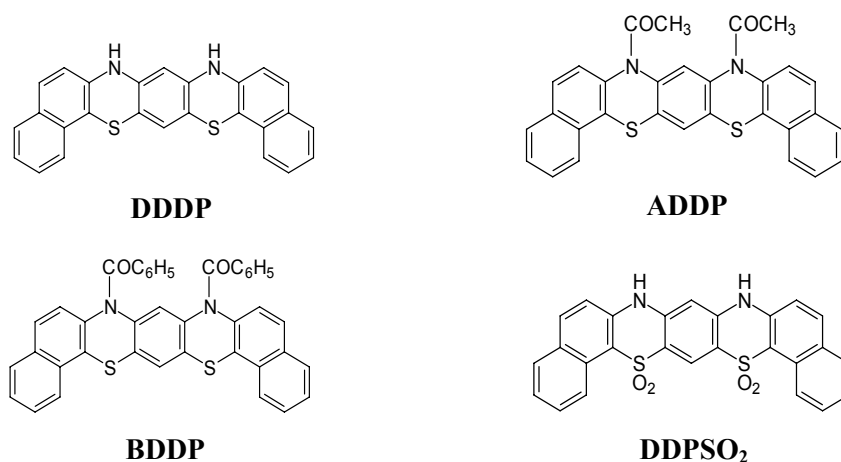
The dibenzo-diaza-dithia-pentacene derivatives (see Scheme 1), 16*H*,18*H*-dibenzo[*c,l*]-7,9-dithia-16,18-diazapentacene (DDDP), dibenzo[*c,l*]-16,18-diacetyl-7,9-dithia-16,18-diazapentacene (ADDP), dibenzo[*c,l*]-16,18-dibenzoyl-7,9-dithia-16,18-diazapentacene (BDDP) and 16*H*,18*H*-dibenzo[*c,l*]-7,9-dithia-16,18-diazapentacene-7,7,9,9-bis-

dioxide (DDPSO<sub>2</sub>), were synthesized and characterized according to previously published procedures.<sup>15,16</sup> The highly chlorinated phenothiazine derivatives (see Scheme 2), 1,1,2,2,3,3,4,6,7,8,9-undecachloro-1,2-dihydro-phenothiazine (UDP), 1,2,3,4,6,7,8,9-octachloro-phenothiazinyl (OCP), 1,2,4,6,7,8,9-heptachloro-3-hydroxy-phenothiazine (HHP) and 1,2,4,6,7,8,9-heptachloro-3*H*-phenothiazine-3-one (HPO), were synthesized and characterized according to previously published procedures.<sup>17,18</sup>

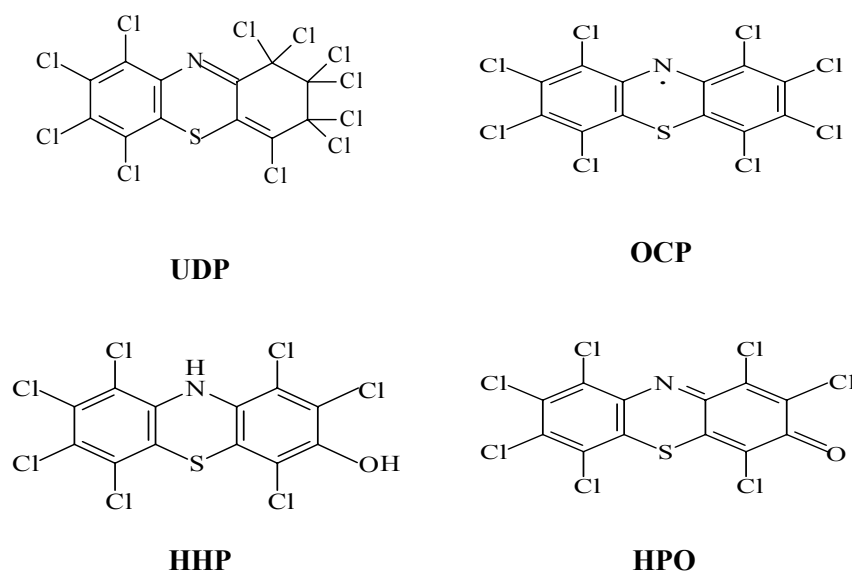
The modified graphite electrodes were obtained by using the method described elsewhere.<sup>19-21</sup> Cyclic voltammetric (CV) measurements were carried out in a conventional three-electrode electrochemical cell. A saturated calomel electrode (SCE) and a coiled Pt wire served as reference and counter electrode, respectively. The cell was connected to a computer-controlled voltammetric analyzer (Autolab-PGSTAT10, Eco Chemie, Utrecht, Netherlands). The supporting electrolyte was a 0.1 M phosphate buffer (pH = 7).

MO calculations were performed, regarding  $\sigma$  and  $\pi$  electrons, on the four DDDP derivatives and the four Ph-Cl derivatives, for their neutral species and ions (both positive and negative). Computations were made at the restricted Hartree-Fock (RHF) level for the closed-shell molecules and in the unrestricted Hartree-Fock approximation (UHF) for radical species (such as OCP or ionized forms). Two semi-empirical SCF MO methods were used: Austin Model 1, AM1<sup>22</sup> and the Parametric Model number 3, PM3,<sup>23</sup> by means of HyperChem 7.5 software package.<sup>24</sup> The two methods are based on the Neglect of Differential Diatomic Overlap (NDDO) integral approximation, and differ by their parameterization. The computation options were: total charge: 0, +1 or -1; spin multiplicity: 1 (singlet) and 2 (doublet) for radicals, state: lowest. The geometries of the molecules were optimized by the Polak-Ribiere (conjugate gradient) algorithm approach. The SCF convergence limit was of 0.042 kJ/mol, and RMS gradient was of  $4.18 \cdot 10^9$  kJ/(m $\times$ mol).

For the optimized geometry of the molecules, the energy related parameters (total electronic energies, enthalpies of formation, energies of frontier orbitals: HOMO and LUMO), electron distributions (charge densities and bond orders), and the geometrical parameters (bond lengths, bond angles, molecular areas)<sup>24</sup> were calculated. The same quantities were calculated for their positive and negative radical-ions. Since for these species the UHF approximation was applied, one-electron orbital energies are obtained (different for the  $\alpha$ - and  $\beta$ -spin electron). Therefore, LUMO energy for instance is here the energy of the lowest "empty" one-electron energy level.



Scheme 1 – Structures of the dibenzodiazadithiapentacene derivatives.



Scheme 2 – Structures of the highly chlorinated derivatives of phenothiazine.

Both (AM1 and PM3) computational methods gave quite similar results for the geometry of the molecules and their parameters. For the sake of clarity, only the results of PM3 calculations will be presented in the following.

## RESULTS AND DISCUSSION

### Electrochemical behavior of adsorbed compounds

The formal redox potentials,  $E^0$ , (estimated as the average of cathodic and anodic peak potentials) for DDDP and Ph-Cl compounds (Table 1) were

placed at 260-290 mV vs. SCE for DDDP derivatives and around 0 mV vs. SCE for Cl-Ph derivatives (at pH 7). For each electrode, the surface coverage ( $\Gamma$ , mol cm<sup>-2</sup>) was estimated from the under peak areas, recorded during the CV measurements at low scan rate ( $v < 10$  mV s<sup>-1</sup>), and considering the surface redox valency equal to 1.<sup>25</sup>

Using the relations (1) and (2),<sup>26</sup> from the variation of the peak potentials with the potential scan rate, the heterogeneous electron transfer rate constants ( $k_s$ , s<sup>-1</sup>) for DDDP and Ph-Cl compounds redox process were calculated (Table 1).

Table 1

Electrochemical parameters corresponding to voltammetric response of DDDP and Ph-Cl derivatives adsorbed on graphite. Experimental conditions: see Fig. 1

| Compound           | $E^0$ (mV vs. SCE) | $k_s$ (s <sup>-1</sup> ) | $k_{\text{deact}}$ (mol cm <sup>-2</sup> s <sup>-1</sup> ) | $\Gamma$ (nmol cm <sup>-2</sup> ) | Reference |
|--------------------|--------------------|--------------------------|--|-----------------------------------|-----------|
| DDDP               | 280                | 30.1                     | 1.8  | 0.6                               | 19        |
| ADDP               | 268                | 21.7                     | 1.9  | 0.4                               |           |
| BDDP               | 263                | 167.6                    | 0.2  | 0.2                               |           |
| DDPSO <sub>2</sub> | 253                | 40.2                     | 0.4  | 0.4                               |           |
| UDP                | -15                | 9.5                      | 9.0  | 2.2                               | 20        |
| OCP                | -9                 | 7.9                      | 15.2   | 3.1                               | 21        |
| HHP                | -4                 | 13.4                     | 46.8   | 3.9                               |           |
| HPO                | -11                | 4.4                      | 28.2   | 5.8                               |           |

$$E_{\text{pc}} - E^0 = \frac{2.3RT}{\alpha nF} * \lg \frac{\alpha nF}{RTk_s} + \frac{2.3RT}{\alpha nF} * \lg v \quad (1)$$

$$E_{pa} - E^0 = \frac{2.3RT}{(1-\alpha)nF} * I_g \frac{(1-\alpha)nF}{RTk_s} + \frac{2.3RT}{(1-\alpha)nF} * I_{gv} \quad (2)$$

where:  $k_s$  [ $s^{-1}$ ] is the heterogeneous electron transfer rate constant;  $\alpha$  is the transfer coefficient;  $n$  is the number of transferred electrons; the other parameters have the usual significance.

Stability tests were done by cycling the electrode potential within the range covering the electrochemical activity of compound and the resulting voltammograms were recorded. The time dependence of the surface coverage decrease showed that this process obeys a zero order kinetics and, therefore, the deactivation rate constants were determined from the slope of this dependence.<sup>19-21</sup>

### MO estimations of electrochemical parameters

In order to evaluate the adsorption of these molecules on the graphite surface, the molecular

contact surface with the solid was estimated. A parallel orientation of the molecular plane (for DDDP derivatives of the plane of the three central rings) to the solid surface was assumed, and the projection of the molecular surface on the graphite surface was calculated, using optimized geometries of the molecules (computed by the HyperChem software) and usual van der Waals radii of the atoms. From these surfaces  $A_{pr}$  (Table 2) the respective surface coverages for a compact adsorption were obtained ( $\Gamma_{calc}$ ). As concerns DDDP-derivatives, except for the last molecule, there is a good linear correlation between these values and experimental  $\Gamma_s$  values and their order of magnitude suggested a monolayer surface coverage.

Table 2

Calculated (PM3) properties for the investigated molecules

| Property                                 | DDDP   | ADDP   | BDDP   | DDPSO <sub>2</sub> | UDP    | OCP    | HHP    | HPO    |
|--|--------|--------|--------|--------------------|--------|--------|--------|--------|
| $\Delta H_f$ (kcal/mol)                  | 135.3  | 67.5   | 137.2  | 14.7               | 16.5   | 62.4   | -17.9  | 20.4   |
| $A_{pr}$ ( $\text{\AA}^2$ )              | 129    | 146.6  | 185.5  | 130.6              | 106.8  | 106.1  | 104.3  | 100.3  |
| $\Gamma_{calc}$ (nmol.cm <sup>-2</sup> ) | 0.13   | 0.11   | 0.09   | 0.13               | 0.16   | 0.16   | 0.16   | 0.17   |
| Polarizability ( $\text{\AA}^3$ )        | 49.27  | 56.78  | 72.43  | 65.55              | 44.85  | 38.77  | 37.80  | 37.03  |
| $\epsilon_{LUMO}$ (eV)                   | -0.868 | -0.997 | -0.996 | -1.245             | -1.945 | -1.852 | -1.035 | -2.358 |
| $\epsilon_{HOMO}$ (eV)                   | -7.664 | -8.064 | -8.258 | -8.935             | -8.992 | -8.171 | -8.323 | -8.940 |
| $X_V$ (eV)                               | 4.266  | 4.530  | 4.627  | 5.090              | 5.468  | 5.012  | 4.679  | 5.649  |
| $\epsilon_{LUMO}(A^+)$ (eV)              | -4.496 | -4.682 | -4.751 | -4.902             | -5.757 | -6.857 | -4.897 | -5.863 |
| $\epsilon_{LUMO}(A^+)$                   |        |        |        |                    |        |        |        |        |
| opt. geom. (eV)                          | -4.502 | -4.648 | -4.635 | -4.795             | -5.871 | -6.823 | -4.979 | -5.888 |
| $\Delta H_+$ (kcal/mol)                  | 163.3  | 172.0  | 175.7  | 194.9              | 192.4  | 165.6  | 176.4  | 190.3  |
| $\Delta H_+$ (kcal/mol)                  | 155.2  | 163.0  | 170.2  | 177.1              | 188.3  | 158.2  | 168.0  | 184.3  |
| opt. geom.                               |        |        |        |                    |        |        |        |        |

$\Delta H_f$  = enthalpy of formation;  $A_{pr}$  = projected area;  $\epsilon$  = orbital energies;  $X_V$  = vertical electronegativity;  $\Delta H_+$  = ionization energy.

On the other hand, for Ph-Cl derivatives the surfaces are very close to each other (between 100.3  $\text{\AA}^2$  for HPO and 106.8  $\text{\AA}^2$  for UDP) and so are the calculated surface coverages,  $\Gamma_{calc}$ : from  $1.56 \cdot 10^{-10}$  mol cm<sup>-2</sup> (UDP) to  $1.66 \cdot 10^{-10}$  mol cm<sup>-2</sup> (HPO). While there is a significant correlation with the experimental values ( $r = 0.98$ ), the calculated values don't account for the large differences

between the experimental coverage. Taking into account the average value ( $\sim 5$ ) of the graphite surface roughness,<sup>27</sup> it could be presumed that the saturation adsorption points to a multilayer surface coverage for the all chlorinated phenothiazine derivatives.

Neutral species and ions (both positive and negative) were considered and heats of formation,

energies of frontier orbitals (HOMO and LUMO), and polarizabilities were calculated. The energies for positively ( $\Delta H_+$ ) and negatively ionization of the molecules were estimated as differences between the energies of the ionic and neutral species, using for the ion both its optimized geometry (as estimations of the adiabatic ionization potential  $IP_{ad}$ , and of the adiabatic electron affinity  $EA_{ad}$ , respectively) and the unchanged geometry of the neutral species (the vertical ionization potential or electron affinity, estimations of the activation energy). Some of these quantities are given in Table 2.

No quantitative correlations are to be expected between these calculated values and the experimental data, since the effects of adsorption and of solvation were not considered. The number of compounds in a series is also too small, to allow for relevant conclusions. Nevertheless, there is a relation between the formal redox potentials,  $E^0$ , and the LUMO energy in the positively ionized compounds. The Ph-Cl derivatives, with lower redox potentials present also lower LUMO energies (Fig. 1A). The overall correlation coefficient  $r$  is somewhat better for the optimized geometry of the cation (0.864 – AM1; 0.809 – PM3) than for unchanged geometry (0.85 – AM1; 0.76 – PM3),

but significant in all cases. A significant linear correlation was found for DDDP-derivatives ( $r \sim 0.95 - 0.99$ ), while for Ph-Cl derivatives, whose  $E^0$ -values are poorly differentiated, no correlation is evident. For DDDP derivatives the expected negative correlation between  $E^0$ -values and the energy difference  $\Delta H_+$  with consideration of the cation optimized geometry (the adiabatic ionization potential) is quite strong ( $r$ -value of  $-0.949$  for the average of AM1 and PM3 calculations), while for Ph-Cl derivatives their value is only  $-0.704$ .

Another useful parameter in the discussion of relative reactivity in electron transfer reactions is the vertical electronegativity,  $X_v$ ,<sup>11-13</sup> defined as:

$$X_v = \frac{1}{2} (IP + EA) \quad (3)$$

or, taking into account Koopman's theorem:

$$X_v = - \frac{1}{2} (\epsilon_{HOMO} + \epsilon_{LUMO}) \quad (4)$$

where  $\epsilon$  are the orbital energies. The values, also given in Table 2, correlate quite well with the  $E^0$ -values for the DDDP derivatives, especially for the PM3 approximation ( $r = -0.973$ ), while for the Ph-Cl derivatives  $r = -0.846$  (Fig. 1B).

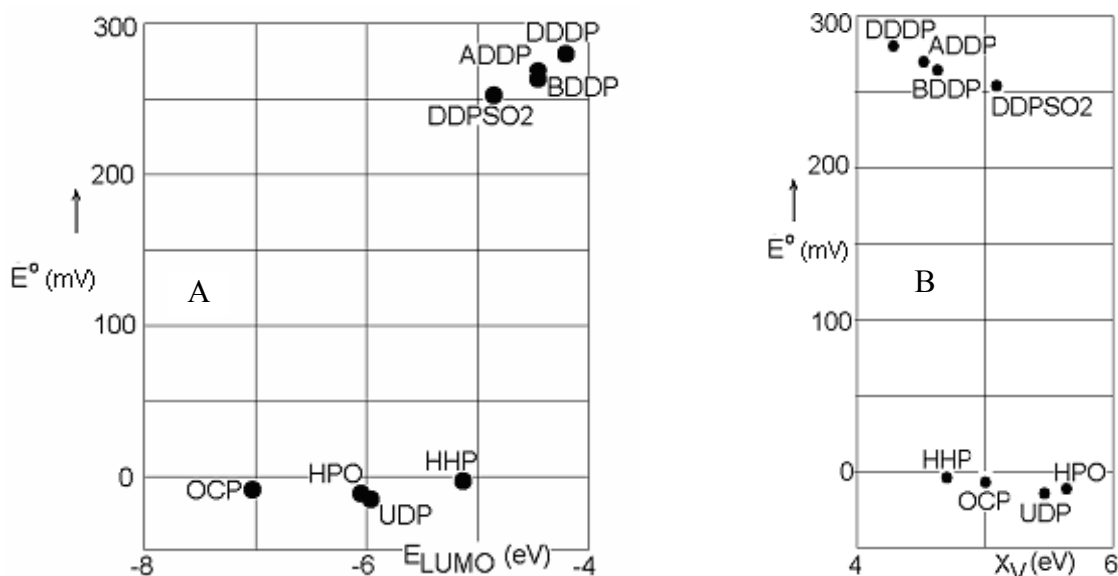


Fig. 1 – Experimental formal redox potentials,  $E^0$  versus calculated (AM1) LUMO energies of the positive ions (A) and versus calculated (PM3) vertical electronegativities (B). Experimental conditions: starting potential, 0 mV vs. SCE (DDDP compounds) and -200 mV vs. SCE (Ph-Cl compounds); scan rate, 50 mV s<sup>-1</sup>; supporting electrolyte, 0.1 M phosphate buffer solution, pH 7; surface coverage,  $\sim 3 \cdot 10^{-10}$  mol cm<sup>-2</sup> (DDDP compounds) and  $\sim 4 \cdot 10^{-9}$  mol cm<sup>-2</sup> (Ph-Cl compounds).

The heterogeneous electron transfer rate constant ( $k_s$ ) appears to be positively correlated to the polarizability of the molecules. BDDP actually

shows the highest polarizability. The correlation coefficient is about 0.8 for all the eight compounds (the correlation is significant at  $p < 0.05$  for

r-values above 0.7), but for Ph-Cl derivatives taken separately there is no correlation. The correlation is about the same with the quantities related to polarizability, such as the molecular volume, the molecular surface or the projected surface  $A_{pr}$ .

The deactivation rate constants  $k_{deact}$  present a significant correlation to the polarizability of the eight compounds ( $r \sim -0.74$ ), better again for the DDDP-type compounds ( $r \sim -0.92$ ) than for the Ph-Cl derivatives ( $r \sim -0.69$ ).

## CONCLUSIONS

A correlation between the electrochemical parameters and structural and molecular characteristics for four phenothiazine derivatives containing two linearly condensed phenothiazine moieties (DDDP derivatives) and four highly chlorinated phenothiazine derivatives (Ph-Cl derivatives) has been realized.

Calculated molecular surfaces proved valuable for the characterization of adsorption patterns, while  $E^{0'}$ ,  $k_s$  and  $k_{deact}$  values could be correlated to electronic characteristics of the molecules, as calculated by semi-empirical MO methods (AM1 and PM3).

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