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Research Article

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Food dyes as blood cell dyes: An experimental and molecular modelling study to brilliant blue FCF and brown HT

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Abstract In this work, the food dyes brilliant blue FCF and brown HT are experimentally tested as blood cell dyes. Quantum chemical calculations by Semi-Empirical (PM6) method were also performed. Calculated HOMO and LUMO orbitals energies, dipole moments, polar surface areas and entropies, suggested a higher affinity by the cells towards $[\text{blue CFC}]^{-2}$ in comparison with $[\text{brown HT}]^{-2}$. For both species, protonation of the benzene central ring (by NH_3^+ of amino acids present at the plasmatic membranes) is supposed based on the specific location of LUMO orbitals.

Keywords Brilliant Blue FCF, Brown HT, Blood cells, Molecular modelling; Semi-Empirical

Introduction

The classical technique of light microscopy remains the basis of any cell study and, consequently, the use of several dyes to promote the appropriate visualisation of the cells and their organelles, is paramount [1,2].

Brilliant blue FCF(ethyl-[4-[[4-[ethyl-[(3-sulfophenyl) methyl] amino] phenyl]-(2-sulfophenyl) methylidene]-1-cyclohexa-2,5-dienylidene]-[(3-sulfophenyl)methyl]azanium), CAS Registry Number: 3844-45-9**,** it is a colorant used in food (such as in ice pops or in the liqueur blue curaçao) and cosmetic (such as in shampoos and mouthwashes) industries. It is water soluble, with a λ_{max} at about 628 nm. It is generally a disodium salt with chemical formula $C_{37}H_{34}N_2Na_2O_9S_3$ (Figure 1).

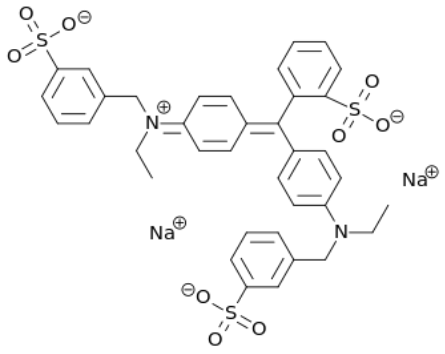


Figure 1: Structural formula for brilliant blue FCF

Brown HT (Figure 2), Disodium 4-[(2E)-2-[(5Z)-3-(hydroxymethyl)-2,6-dioxo-5-[(4-sulfonatonaphthalen-1-yl)hydrazinylidene]-1-cyclohex-3-enylidene]hydrazinyl]naphthalene-1-sulfonate, $C_{27}H_{18}N_4Na_2O_9S_2$, is a synthetic coal tar diazo food dye, used, for example, to substitute cocoa or caramel as a colorant.

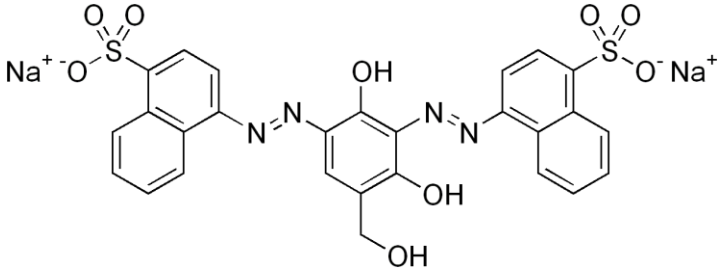


Figure 2: Structural formula for brown HT

Certainly, in cytology, the use of low cost and/or low toxicity dyes is important, taking into account the green chemistry tendencies. In this connection, in the present work, brilliant blue FCF and brown HT are tested as blood cell dyes.

Experimental

Quantum chemical calculations were performed by using Spartan´14 (version 1.1.8) [3]. A semi-empirical (PM6) method was used. Since both dyes are disodium salts that dissociate into solution, in both cases the calculations were performed on the dianions: $[C_{37}H_{34}N_2O_9S_3]^{-2}$ and $[C_{27}H_{18}N_4O_9S_2]^{-2}$, respectively.

The dyes solutions were prepared as follows: 0.028 g of the dyes were dissolved in 250, 500, 750 and 1,000 μL of methanol (Vetec).

The blood laminas for microscopy were prepared by the following protocol: blood smears on the glass plaque were dried at room temperature (26 ºC) for 1 h. After that, the blood smears were covered with the dyes solutions (20 drops) for 3 minutes. Hence, 20 drops of distilled and buffered water were used (10 minutes of contact) in order to remove the dye solution excesses. Finally, the microscopy laminas were washed with distilled water and dried at room temperature (26 ºC) at vertical position, for 30 minutes.

The laminas were then visualized (and photographed) in a binocular optical microscope (Olympus, model CX 21; 10 X and 100 X objective lenses, that is, a 1,000 X of maximum magnification).

Results and Discussion

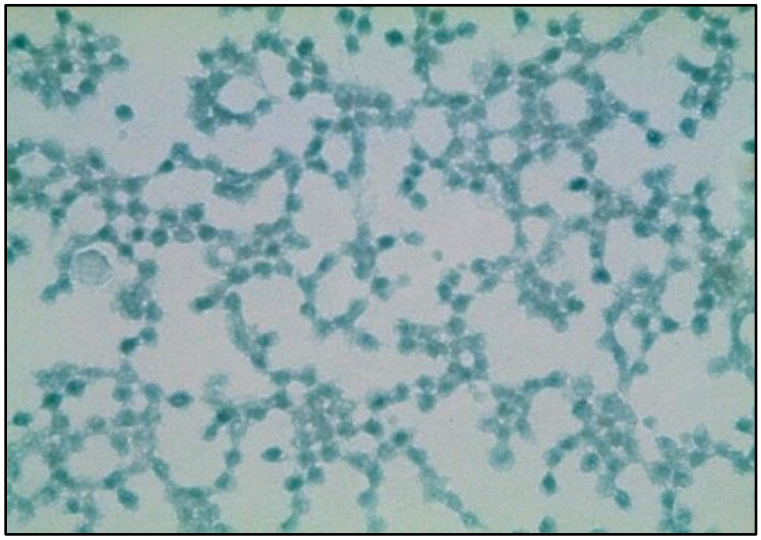


Figure 3: Blood cells (100 X) with brilliant blue FCF (0.028 g/1,000 μL.)

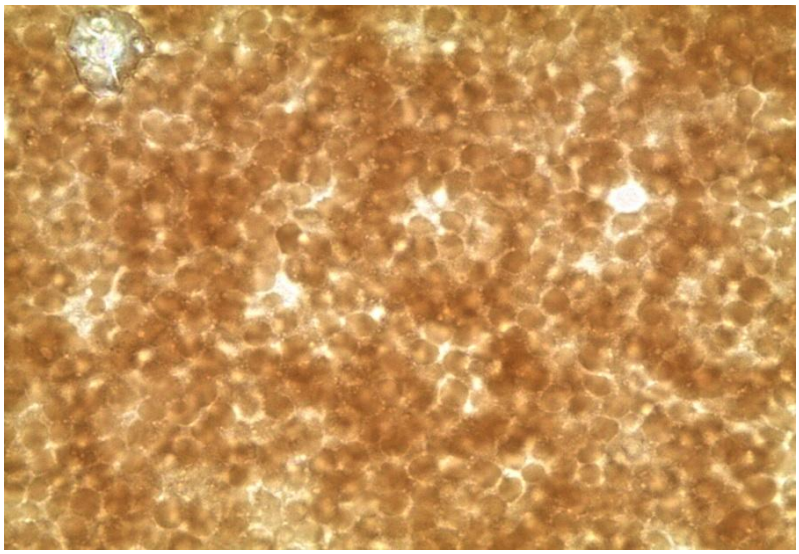
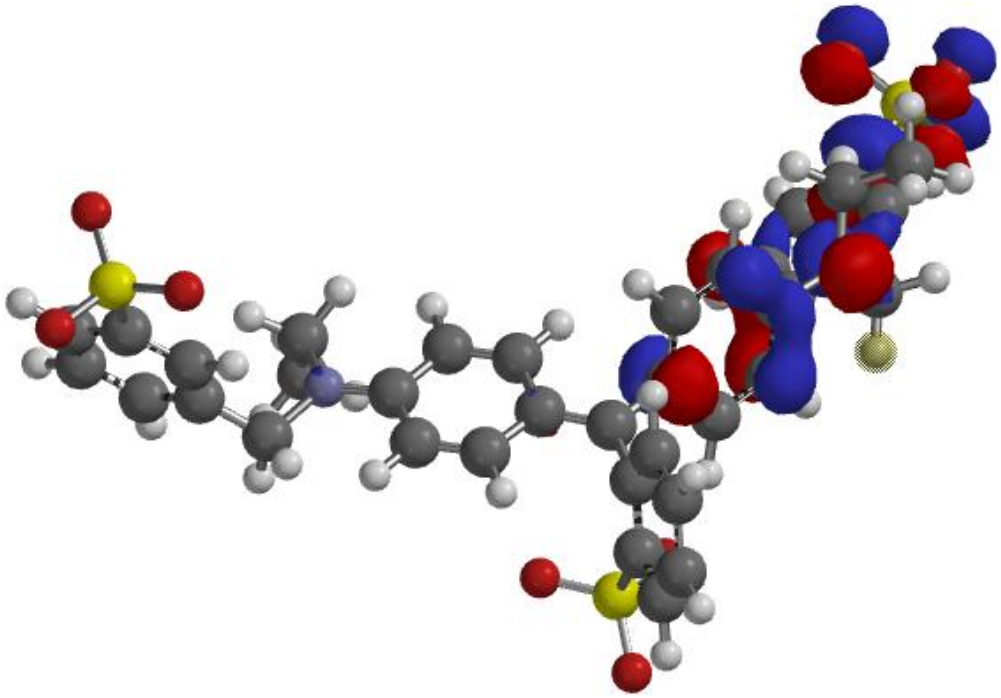


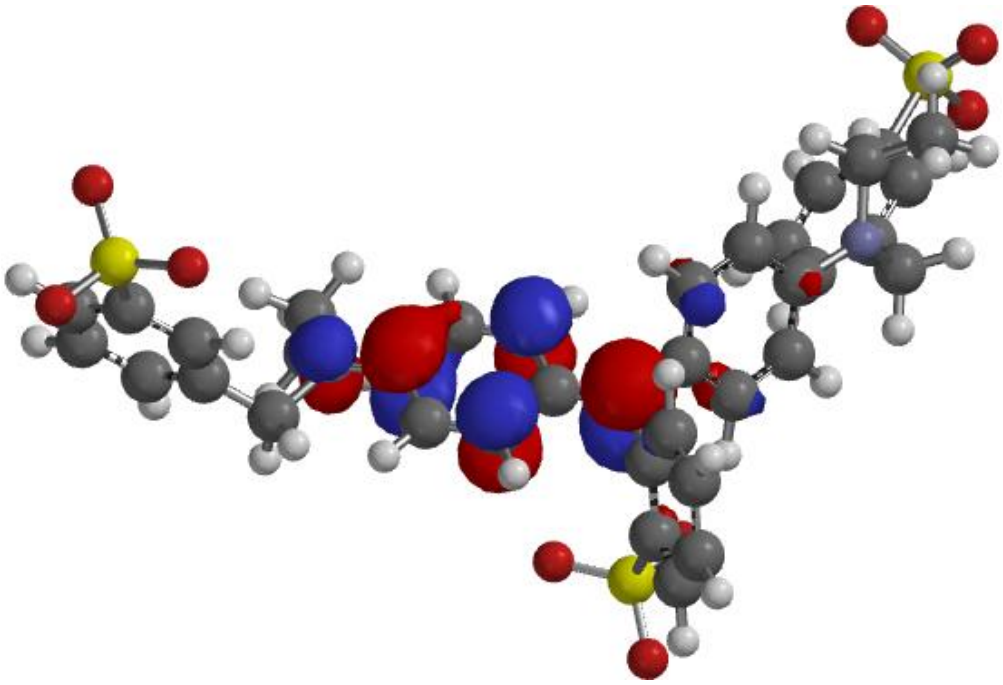
Figure 4: Blood cells (1,000 X) with brown HT (0.028 g/1,000 µL)

It was verified that brilliant blue FCF and brown HT exhibit very high affinity towards plasmatic membranes, specially the solutions with the concentrations 0.028 g/750 µL and 0.028 g/1,000 µL. Two illustrative results are shown in Figures 3 and 4.

Brilliant blue is a basic dye. Hence, it is supposed that it must interact with the NH_3^+ groups of the amino acids present at the plasmatic membranes of the blood cells. On the other hand, it was verified that both dyes do not have any specific affinity, that is, for withe or red blood cells, for example. The interaction with NH_3^+ groups occurs, probably, by protonation of the respective dianions.

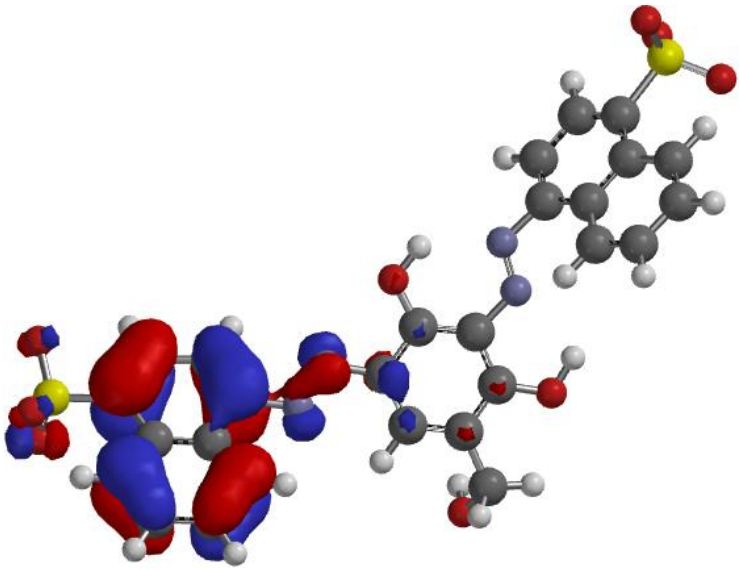


(a)

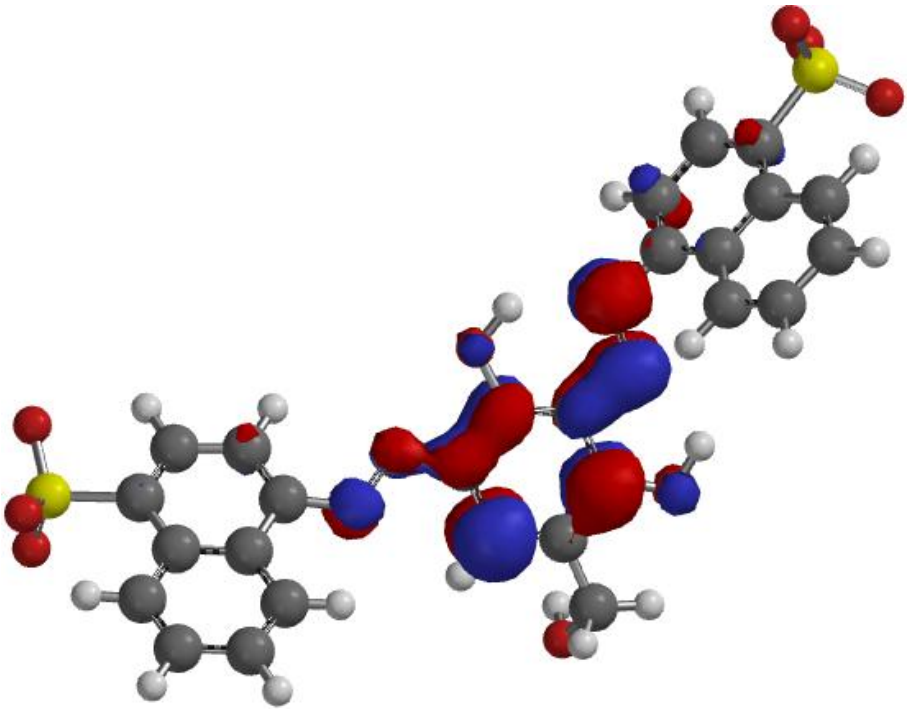


(b)

Figure 5: HOMO (a) and LUMO (b) orbitals for[blue FCF]$^{-2}$



(a)



(b)

Figure 6: Homo (a) and LUMO (b) orbitals for [brown HT]$^{-2}$

The quantum chemistry calculations results are summarized in Table 1. As can be verified, the [brown HT]$^{-2}$ exhibits a higher dipole moment and a large polar surface area than [blue CFC]$^{-2}$. So, it could be supposed a lower capacity of [brown HT]$^{-2}$ to penetrate the lipid cell barrier [4], in comparison with [blue CFC]$^{-2}$. Furthermore, [brown HT]$^{-2}$ exhibits a LUMO orbital with an 1.77 eV energy, whereas the same orbital to [blue CFC]$^{-2}$ has a 0.44 eV energy. So, since the interaction with the NH_3^+ groups could be supposed as occurring by protonation, such protonation process is most favourable to [blue CFC]$^{-2}$. Once again, the calculated data pointed out to a [blue CFC]$^{-2}$ higher affinity towards cell bloods in comparison with [brown HT]$^{-2}$. The HOMO and LUMO orbitals for both dianions are shown in Figures 5 and 6.

Table 1: Calculated quantum chemical parameters for blue FCF and brown HT dianions

Parameter	**$[C_{37}H_{34}N_2O_9S_3]^{-2}$**	**$[C_{27}H_{18}N_4O_9S_2]^{-2}$**
Energy/kJmol^{-1}	-1012.13	-1034.08
E_{HOMO}/eV	-5.22	-4.95
E_{LUMO}/eV	0.44	1.77
Volume/A^3	697.60	523.31
Area/A^2	709.9	545.35
PSA/A^2	154.83	183.59
Ovality	1.87	1.74
Polarizability	96.63	82.24
ZPE/kJmol^{-1}	1675.17	1034.43
Dipolemoment/D	15.45	23.86
H^o/au (298.15 K)	0.2919	0.0310
S^o/Jmol^{-1} (298.15K)	898.04	763.00
G^o/au (298.15K)	0.1899	-0.0556

As can be verified in Figure 5, the LUMO for [blue CFC]$^{-2}$ is located, essentially, on the central benzene ring. Hence, it is possible to suppose a protonation of such ring by the NH_3^+ group, as a main step in the interaction with

the amino acids groups of the plasmatic membrane. So, it is also possible to suppose that the Na^+ cations remain as the counter-ions of the SO_3^- groups, as the dye molecule penetrates the lipidic barrier.

As can be verified in Figure 6, to $[brown\ HT]^{-2}$ the LUMO orbital is also located, mainly, on the central benzene ring. So, the same reasoning made to $[blue\ CFC]^{-2}$ can be pointed out.

Since the ovality of both dianions are very similar (Table 1), it could be concluded that steric hindrance is not, for $[blue\ CFC]^{-2}$ and $[brown\ HT]^{-2}$, a factor to be considered for comparison and prediction of their affinities towards the cell membranes.

The calculated standard entropy values for $[blue\ CFC]^{-2}$ and $[brown\ HT]^{-2}$, 898.04 and 763.00 $Jmol^{-1}$, respectively, also suggests a higher affinity by the cells towards $[blue\ CFC]^{-2}$, since it is most favourable to the cell "incorporate" in its structure a molecule (dye) with higher entropy.

References

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