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Antioxidant activity of ionic liquids formed by carnitine and food acids: a correlation between the data of their FRAP assay and their pK_a values

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Abstract. The usage of the restricted open-shell Hartree-Fock (ROHF) method to the calculation of electronic structures of the complex Fe(III) with 2,4,6-tripyridyl-s-triazine (TPTZ): $[Fe(III)(TPTZ)_2]^{3+}$ and its adduct with the ionic pair $[Carnitine\text{-}H]^+$ $[gentisic\ acid\ anion]^-$ (formed by a protonated carnitine zwitterion and an anion of gentisic acid) allows one to reveal that the anion entering this pair retains its ability to quench free radicals. Antioxidant activity of the studied ionic liquids in their reaction with the complex $[Fe(III)(TPTZ)_2]^{3+}$ is determined by the food acids anions included in their ionic pairs. It is also shown that the values of the antioxidant activity of the studied ionic liquids defined by the FRAP assay correlate with the pK_a values of their precursor food acids.

Keywords: antioxidant activity, ionic liquids, carnitine, food acids, the FRAP assay, the ROHF method.

Activitatea antioxidantă a lichidelor ionice formate din carnitină și acizi alimentari: o corelație între datele testului FRAP și valorile pK_a

Rezumat. Utilizarea metodei Hartree-Fock cu înveliș deschis (ROHF) pentru calcularea structurilor electronice ale complexului Fe(III) cu 2,4,6-tripiridil-s-triazină (TPTZ): $[Fe(III)(TPTZ)_2]^{3+}$ și aductul său cu perechea ionică $[Carnitină\text{-}H]^+$[anionul acidului gentisic]$^-$ (formată dintr-un zwitterion carnitină protonată și un anion al acidului gentisic) permite să se evidențieze faptul că anionul care intră în această pereche își păstrează capacitatea de a stinge radicalii liberi. Activitatea antioxidantă a lichidelor ionice studiate în reacția lor cu complexul $[Fe(III)(TPTZ)_2]^{3+}$ este determinată de anionii acizilor alimentari incluși în perechile lor ionice. De asemenea, se arată că valorile activității antioxidante a lichidelor ionice studiate, definite prin testul FRAP, se corelează cu valorile pK_a ale acizilor alimentari precursori.

Cuvinte-cheie: activitate antioxidantă, lichide ionice, carnitină, acizi alimentari, testul FRAP, metoda ROHF.

1. Introduction

The present work is devoted to the quantum-chemical study (by the ROHF method) of antioxidant activity of ionic liquids formed due to the interaction of a protonated carnitine form with anions of some food acids (see the work [1]). The above antioxidant activity was determined by means of the ferric reducing antioxidant power (FRAP) assay which is based on the reaction of the investigated compounds with the complex $[Fe(III)(TPTZ)_2]^{3+}$. In the works [2], [3], it was shown that the interaction of food acid molecules with the carnitine zwitterion occurs with the participation of the tautomeric form of carnitine, which has a higher energy. In this case (after the transfer of protons from acid molecules to carnitine zwitterions), in the resulting ionic pairs, the acid anions are connected with the protonated carnitine cations via the oxygen atom belonging to the hydroxyl group of carnitine.

As it was also shown in the work [3] in resulting ionic pairs formed by carnitine and food acids anions the latter bind to the protonated carnitine cation through the oxygen atom belonging to their ionized hydroxyl groups. On the side of the protonated carnitine zwitterion, its hydroxyl group, containing a proton that transforms the carnitine zwitterion into the cation in question, participates in the formation of the above ionic pairs. Previously, in the work [4], it was established that the $ABTS^{+\bullet}$ radical cation interacts exclusively with the anions of food acids (but not with their neutral molecules).

Thus, since the ionic pairs of the studied ionic liquids consist of a protonated carnitine cation and acidic anions, then, as it was also shown in [3], the anions of these ionic pairs can interact with the $ABTS^{+\bullet}$ cation radical. However, the interaction of these anions with the $ABTS^{+\bullet}$ cation radical can be achieved in two ways (see [3]): firstly, by means of the interaction with these anions after the dissociation of ion pairs in solution; and, secondly, due to the interaction with these anions remaining in the structure of the corresponding ion pairs. In the first case, the number of anions (and, consequently, the antioxidant activity EC50) should be determined (correlated) with the pK_a values of the unionized acids. However, as shown in the work [3], ion pairs are also capable of demonstrating an antiradical effect, quenching free radicals.

Further, since the cation-radical $ABTS^{+\bullet}$ is an organic compound, we were interested in investigating the antiradical effect of ionic pairs formed by protonated carnitine and anions of food acids on inorganic compounds containing unpaired electrons. This is precisely what we accomplished in this study, using the interaction of the ion pair protonated carnitine and the gentisic acid anion with complex $[Fe(III)(TPTZ)_2]^{3+}$ as an example.

2. Computational methods

The geometric and electronic structure calculations of all studied molecular systems were performed by the restricted open-shell Hartree-Fock method ROHF using the 6-31G(d) basis set for atomic orbitals. All calculations were performed considering the solvent (water) based on the IEFPCM option of the Gaussian09 software package [5].

3. Results and discussion

The main results of the present work are given in Figures 1–3 and in Table 1. One can easily note that the spatial distribution of the unpaired electron density in the complex $[Fe(III)(TPTZ)_2]^{3+}$ shown in Figure 1 is localized mainly on one of its two end pyridyl groups. At the same time (see Figures 2 and 3) for both the resulting products (which describe the interactions of the complex $[Fe(III)(TPTZ)_2]^{3+}$ with an anion of gentisic acid and with the ionic pair formed by protonated carnitine and an anion of gentisic acid) the electron density of the considered unpaired electron is localized on the structural fragments describing their former gentisic acid anions. Thus, the ionic pairs of the studied ionic liquids retain the antioxidant ability of the anions of the corresponding food acids. The data, presented in Table 1, show that there is a clear-cut correlation between the data of the FRAP assay for antioxidant activity of the studied ionic liquids and the pK_a values, characterizing the precursor food acids, whose anions enter the above liquids.

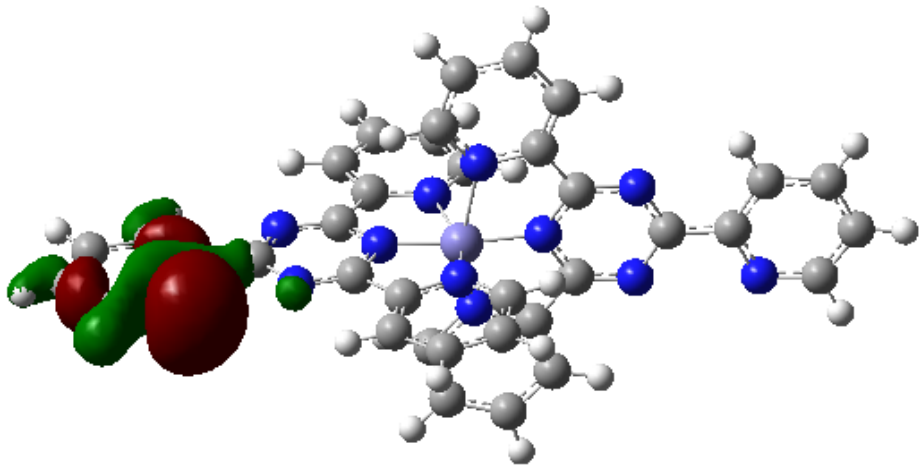

Figure 1. The spatial distribution of the unpaired electron density in the complex $[Fe(III)(TPTZ)_2]^{3+}$

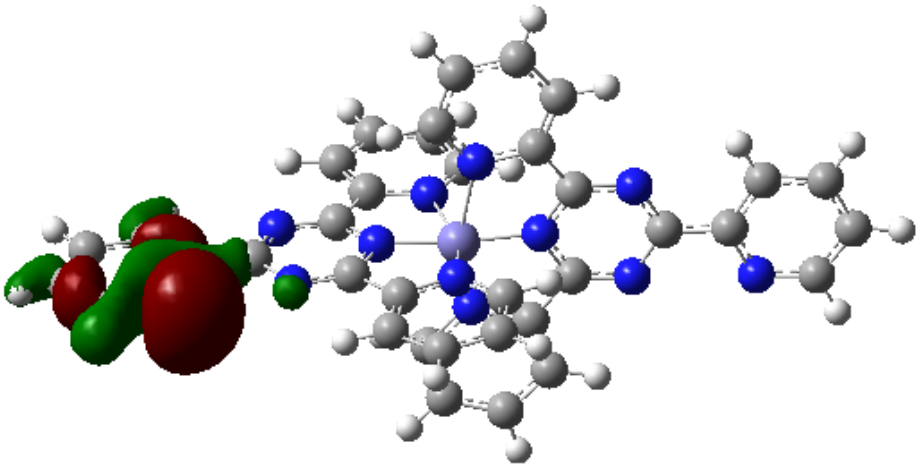

Figure 2. The spatial distribution of the unpaired electron density in the product of the reaction between the complex $[Fe(III)(TPTZ)_2]^{3+}$ and an anion of gentisic acid

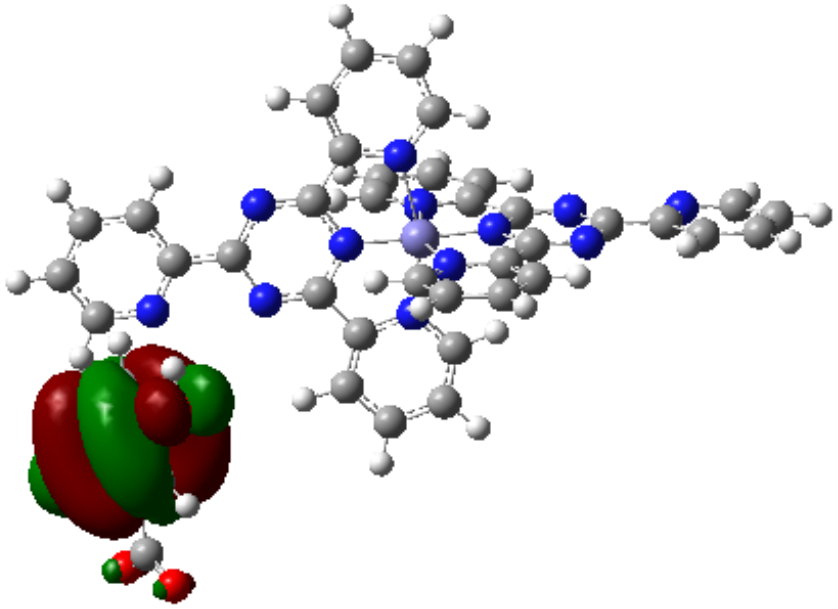

Figure 3. The spatial distribution of the unpaired electron density in the product of the reaction between the complex $[Fe(III)(TPTZ)_2]^{3+}$ and the ionic pair formed by protonated carnitine and an anion of gentisic acid

Table 1. To the correlation of the FRAP antioxidant activities of the studied ionic liquids with the pK_a values of their precursor food acids

Nos.	Ionic Liquid***	FRAP (μmol TE/g)*	pK_a**
1	$[CRN]^+[PRO]^-$	1.01/0.67****	4.49
2	$[CRN]^+[GEN]^-$	2.20/2.03	2.98
3	$[CRN]^+[GAL]^-$	2.08/1.89	3.13
4	$[CRN]^+[SYR]^-$	1.50/1.17	3.93
5	$[CRN]^+[COU]^-$	0.38/0.54	4.64
6	$[CRN]^+[CAF]^-$	0.82/1.08	4.04
7	$[CRN]^+[FER]^-$	1.19/1.49	3.58
8	$[CRN]^+[SIN]^-$	1.33/1.64	3.41

* The data were taken from the work [1]; TE – Trolox equivalents;

** The values of pK_a in water (at T = 293.15–298.15 K and P = 0.1 MPa) were taken from Refs. [6, 7];

*** The abbreviations: $[CRN]^+$, $[PRO]^-$, $[GEN]^-$, $[GAL]^-$, $[SYR]^-$, $[COU]^-$, $[CAF]^-$, $[FER]^-$, and $[SIN]^-$ denote a protonated cation of carnitine and the anions of protocatechuic, gentisic, gallic, syringic, coumaric, caffeic, ferrulic, and sinapic acids, respectively;

**** - FRAP: experimental/calculated by means of Eq. (1).

The regression equation (1), obtained by the least squares method based on the data from Table 1, describes the linear functional relationship between the values FRAP (μmol TE/g) and pK_a:

$$\text{FRAP} = -0.8968\,\text{p}K_a + 4.699; \tag{1}$$

$$R = 0.89; \quad S = 0.31; \quad F = 22.11.$$

Here, R is the multiple correlation coefficient; S is the standard deviation; and F is the F-statistic. The numerical values of R, S, and F given above are high enough to indicate the existence of a linear relationship between FRAP and pK_a. The sign "minus" at the quantity pK_a in equation (1) shows that the *decrease* in pK_a (occurring by passing from one acid to another stronger one) will lead to the corresponding *increase* in the quantity FRAP.

It should be noted here that the inclusion of pK_a as an independent variable in equation (1) is not accidental. Indeed, on the one hand, pK_a describes the acidity of the food

studied acids; however, on the other hand, it can be considered as some measure of the ease with which neutral molecules of these acids can donate protons taking part in different chemical reactions.

So, in Ref. [1], it was shown that ion pairs between the anions of the studied food acids and the protonated zwitterion of carnitine are formed precisely due to the transfer of protons from the molecules of these acids to the zwitterion of carnitine. The lower the pK_a value of an acid (i.e., the stronger the acid), the more easily its proton transfers to the carnitine zwitterion, and, consequently, a more complete separation of the positive and negative charges occurs within the ion pair they form. In turn, a greater negative charge on an acid anion (which forms the ion pair with protonated carnitine) will enhance antioxidant (reductive) activity of this anion when the above ion pair interacts with oxidant molecules or ions containing unpaired electrons.

Obviously, in the media of the studied ionic liquids antioxidant activity of free food acids anions will differ from their activity in the case when these anions enter the ion pairs with protonated carnitine. However, one can expect some significant similarity between the antioxidant activities for the above two kinds of food acids anions.

It should be noted that our ROHF calculations show that the interaction of the ion pairs presented in Table 1 with the $[Fe(III)(TPTZ)_2]^{3+}$ complex cation transforms the food acids anions included in these ion pairs into their corresponding radical derivatives (see Fig. 3). At the same time, the $[Fe(III)(TPTZ)_2]^{3+}$ complex is being reduced and converted into a complex of ferrous iron with TPTZ: $[Fe(II)(TPTZ)_2]^{2+}$. However, as a result of the transformations described above, the protonated zwitterion of carnitine retains its positive charge close to +1 (see below).

Furthermore, since quantum chemical calculations using the ROHF method with the 6-31G(d) basis set often tend to overestimate charge delocalization, we considered it necessary to compare the charge distribution calculated by the ROHF method in molecular systems formed by ion pairs (see Table 1) and the $[Fe(III)(TPTZ)_2]^{3+}$ complex with the results of calculations using the DFT M062X functional, which, firstly, includes electron correlation and, secondly, takes into account corrections for van der Waals dispersion [5]. This was done for the system shown in Figure 3 describing the interaction of an ion pair consisting of protonated carnitine and the gentisic acid anion with the $[Fe(III)(TPTZ)_2]^{3+}$ complex. The numerical results of the calculations for the comparison described above are presented below as the ratio of the total charges calculated by these methods (ROHF/DFT M062X) for various parts of the system shown in Figure 3. The ratio of the total charges on:

- the protonated carnitine zwitterion: 0.894/0.835;
- the radical derivative of gentisic acid: 0.065/0.069;

- the $[Fe(II)(TPTZ)_2]^{2+}$ complex of ferrous iron: 2.041/2.096.

Thus, it can be seen that the ROHF method quite adequately describes the charge distribution in molecular systems resulting from the interaction of the studied ion pairs with the $[Fe(III)(TPTZ)_2]^{3+}$ complex.

4. Conclusion

Thus, based on the results obtained, it can be concluded that the antioxidant activity of the studied ionic liquids in their reaction with the ferric complex $[Fe(III)(TPTZ)_2]^{3+}$ is determined by both the dissociated and undissociated ionic pairs that form these ionic liquids and, in both these cases, the anions of food acids (either free or included in the structure of the ion pairs) interact with the ferric complex $[Fe(III)(TPTZ)_2]^{3+}$ reducing Fe(III) to Fe(II). It was also revealed a clear cut correlation between the data of the FRAP assay describing the antioxidant activity of the studied ionic liquids and the pK_a values, characterizing the precursor food acids, whose anions enter the molecular structures of the above ionic liquids.

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