Protonation of Nitrofurantoin and Furazidine Molecules in Acidic Media-Molecular Modelling Studies

Abstract
The molecular modeling studies on protonation sites of Nitrofurantoin and Furazidine as well as on the stability of particular protonated forms were performed using quantum chemical MP2 method. Performed calculations show that Furazidine oxygen and nitrogen atoms are better proton acceptors than in Nitrofurantoin, therefore the acidity of the media may differentiate Nitrofurantoin and Furazidine antibacterial activity.

Keywords: Nitrofurantoin; Furazidine; Acidity; Protonation sites; Urinary tract infection; Molecular modelling

Introduction
According to literature data in nitrofuran derivatives the major role in antimicrobial activity plays the acidity of physiological medium in urinary tract [1]. It was pointed out [2] that dissociation of Furazidine is hampered in the presence of ascorbic acid (vitamin C). The role of any acidifying agent like ascorbic acid is to prevent alkalization of infected urine and preserve pH close to 5.5 in which Furazidine molecule stays in non-dissociated form what enhances its antibacterial activity [1]. More acidic medium can probably cause better protonation of the Furazidine molecule than of Nitrofurantoin, what can further enhance Furazidine therapeutic efficacy. Therefore studies of possible protonation sites of Nitrofurantoin and Furazidine, along with stability of particular protonated form, can provide valuable estimate for insight into activity determinates of Furazidine moiety, and also for further modification of its structure. Selection of Nitrofurantoin and Furazidine is a wise model because those two compounds differ in two carbon atoms in the rings bridging part of molecules. Moreover there is distinct difference between their antimicrobial activities. In Escherichia coli test they display MIC ≤ 32 µg/ml and 1 µg/ml for Nitrofurantoin and Furazidine, respectively [3], as reported in information leaflets of medicine present on the market.

Methods
All quantum chemical calculations were performed with Spartan 14 V.1.1.4 software package at the MP2/6-31G*/MP2/6-31G* level [4]. The hydration energies were calculated with SM8 model.

Results and Discussion
Calculation on neutral Nitrofurantoin tautomer clearly indicates that N1-H tautomer is the most stable form both in water medium. The same holds for Furazidine molecule.

The neutral Nitrofurantoin and Furazidine molecules can potentially exist in various tautomeric forms. As shown in Tables 1 and 2 the N1-H tautomer of Nitrofurantoin is the most stable. The second most stable tautomer is the one bearing the proton on the O2 nitrogen atom at the MP2/6-31G*/MP2/6-31G* level with hydration energy included Table 1. Calculation of Δ (ΔGo) yields also the O2-H tautomer to be the most stable form after the N1-H tautomer (Table 2). The N1-H tautomer probably does not exist. The attached proton is being transferred from N1 to O2 atom during optimization process yielding the O2-H tautomer.
Table 1: Total MP/6-31G*/6-31G* energies, stabilization energies, ΔG0 and Δ(ΔG0) calculated for neutral and protonated Nitrofurantoin molecule.

| Cation | Total energy | Stabilization energy | ΔG° | Δ(ΔG°) |
|--------|--------------|----------------------|------|--------|
| I (N-H) | -900.956670 | 0.00 | -900.823127 | 0.00 |
| II (N-H) | -900.919856 | 23.10 | -900.777778 | 28.46 |
| III (O-H) | -900.928299 | 17.80 | -900.777699 | 28.51 |
| IV (O-H) | -900.928428 | 17.72 | -900.786946 | 22.70 |
| V (N-H) | -900.936229 | 12.83 | -900.793088 | 18.85 |
| VI (O-H) | -900.869400 | 54.76 | -900.745721 | 48.57 |
| VII (O-H) | -900.873576 | 52.14 | -900.750943 | 48.57 |

| Dication | Total energy | Stabilization energy | ΔG° | Δ(ΔG°) |
|----------|--------------|----------------------|------|--------|
| I (N-H; O-H) | -901.208345 | -157.93 | -901.027358 | -128.15 |
| II (N-H; O-H) | -901.369024 | -258.75 | -901.132061 | -193.86 |
| III (N-H; O-H) | -901.385070 | -267.84 | -901.150271 | -205.28 |
| IV (N-H; N-H) | -901.336768 | -238.51 | -901.116485 | -184.08 |
| V (N-H; N-H) | -901.364274 | -255.77 | -901.129443 | -192.21 |
| VI (N-H; N-H) | -901.385323 | -267.85 | -901.150258 | -205.28 |
| VII (N-H; O-H) | -901.355919 | -250.53 | -901.135881 | -196.25 |
| VIII (N-H; O-H) | -901.315992 | -225.47 | -901.093608 | -169.73 |

Table 2: Total MP/6-31G*/6-31G* energies, stabilization energies, ΔG0 and Δ(ΔG0) calculated for neutral and protonated Furazidine molecule.

| Neutral | Total energy | Stabilization energy | ΔG° | Δ(ΔG°) |
|---------|--------------|----------------------|------|--------|
| I (N-H) | -978.101505 | 0.00 | -977.934728 | 0.00 |
| II (N-H) | -978.047774 | 33.72 | -977.883044 | 32.43 |
| III (O-H) | -978.079351 | 13.90 | -977.896975 | 23.69 |
| IV (O-H) | -978.073821 | 17.72 | -977.898064 | 29.61 |
| V (N-H) | -978.011941 | 56.20 | -977.856693 | 48.97 |
| VI (O-H) | -978.011940 | 56.20 | -977.856734 | 48.94 |

| Dication | Total energy | Stabilization energy | ΔG° | Δ(ΔG°) |
|----------|--------------|----------------------|------|--------|
| I (N-H; O-H) | -978.497706 | -248.62 | -978.249830 | -197.73 |
| II (N-H; O-H) | -978.510679 | -251.15 | -978.274167 | -213.00 |
| III (N-H; O-H) | -978.483563 | -239.74 | -978.230392 | -185.53 |
| IV (N-H; N-H) | -978.501737 | -256.76 | -978.289982 | -292.95 |
| V (N-H; N-H) | -978.501737 | -256.76 | -978.289982 | -292.95 |
| VI (N-H; N-H) | -978.538595 | -274.27 | -978.274167 | -213.00 |
| VII (N-H; O-H) | -978.499000 | -249.43 | -978.249830 | -197.73 |
| VIII (N-H; O-H) | -978.460211 | -225.47 | -978.215781 | -270.60 |
When proton is placed on O₃ oxygen atom then it relocates to one of the atoms of the nitro group. The relative stability of Nitrofurantoin tautomers in water medium is as: N₁-H>O₂-H>O₃-H form. The same trend is observed when the Δ(ΔG₀) values are considered.

In the case of Furazidine molecule also the N₁-H tautomer appeared to be the most stable. Here however the N₃-H and N₅-H tautomers exist. The N₁-H tautomer is stabilized through C-H interaction of more flexible bridging chain with O₁ atom of five membered rings. As in Nitrofurantoin, when proton is placed on O₁ oxygen atom then it relocates to one of the atoms of the nitro group.

In Nitrofurantoin and Furazidine molecules there is 8 potential protonation centers, 3 oxygen atoms and 3 nitrogen atoms and two oxygen atoms of the nitro group (Figure 1). Nevertheless the nitro group, in each of two equivalent resonance structures, can potentially interact via the hydrogen bonding.

Protonation of neutral form yields mostly the other than H-N₁-H+ cations what prevents the change of charge distribution and electrostatic potential pattern around non ionized fragment of neutral molecule believed to be necessary for Furazidine activity.

For Nitrofurantoin molecule the most stable is the N₁-H; N₅-H cation. The N₁-H; O₅-H cation rearranges also to that tautomer (Figure 1). For Furazidine molecule cation N₁-H O₅-H does not exist because it rearranges to N₁-H; O₃-H tautomer. The most stable like in Nitrofurantoin stays the N₁-H; N₅-H form Table 2.

The dication of Nitrofurantoin with the highest stability is the one formed from the most stable N₁-H; N₅-H cation by protonation of O₁ or O₅ oxygen atom. The same holds for Furazidine molecule (Table 1).

Formation of the most stable monocation N₁-H N₅-H is more preferred in Furazidine then in Nitrofurantoin by ca. 7.75 kcal/mole. The most stable dication of Nitrofurantoin is N₁-H; O₁-H; N₅-H, however very close in energy to N₁-H; O₃-H; N₅-H. In Furazidine molecule formation of intramolecular hydrogen bonding yields the N₁-H; O₅-H; N₅-H dication the most stable. This is due to the higher flexibility of the bridging chain, than in Nitrofurantoin.

The nitro group can be protonated at each of the oxygen atoms yielding resonance structure -NOOH+ similar to -COOH [4]. Further protonation of -NO₂ could lead to reduction of -NO₂ yielding the -NH₂ derivative. This is one of the mechanisms that activate Furazidine active substance in the living organism [5,6].

If additional proton is placed on O₃ oxygen of five membered ring of neutral Furazidine, then it relocates to O₃ oxygen atom. It means that formation of cation III is very unlikely. Cation II gets additional stabilization due to intramolecular bonding with one of the oxygen atoms of -NO₂ group. The most stable cation is the N₁-H, N₅-H. The total energy and thermodynamics (ΔG) analysis leads to the same conclusions.

Similar conclusions regarding possible protonation sites appear from analysis of electron charges on the atoms that are eager to accept proton (Table 3).

For neutral Nitrofurantoin and Furazidine nitrogen atoms the most negative is the N₁ atom of the bridging chain. Protonation at this atom leads to the most stable N₁-H; N₅-H cationic form. In the cation the most negatively charge atoms yield the most stable N₁-H; O₁-H; N₅-H dicaticonic form of Nitrofurantoin, and N₁-H; O₅-H; N₅-H dicaticonic form of Furazidine, respectively.

Consequently the N₁-H tautomer of neutral Furazidine is more stable than N₁-H, O₁-H and O₅-H tautomers. Performed calculations show that Furazidine oxygen and nitrogen atoms are good proton acceptors. Therefore it is justified to supplement Furazidine treatment with weak acids, for instance vitamin C, to keep the protonated Furazidine at satisfactory level, preventing urine alkalization. The in vitro studies on Furazidine acidity as function of pH are underway.
Table 3: Electrostatic, Mulliken and natural charges calculated for nitrofurantoin and furazidine at MP/631G*/6-31G* level.

| Atom | Electrostatic | Mulliken | Natural | Electrostatic | Mulliken | Natural |
|------|---------------|----------|---------|---------------|----------|---------|
| N₁   | -0.718        | -0.731   | -0.701  | -0.544        | -0.737   | -0.689  |
| N₂   | -0.009        | -0.444   | -0.375  | -0.229        | -0.371   | -0.310  |
| O₁   | -0.483        | -0.447   | -0.555  | -0.404        | -0.419   | -0.527  |
| O₂   | -0.461        | -0.428   | -0.529  | -0.377        | -0.356   | -0.458  |
| N₃   | -0.314        | -0.195   | -0.237  | +0.215        | -0.340   | -0.228  |
| O₃   | -0.237        | -0.441   | -0.401  | -0.210        | -0.462   | -0.416  |
| N₄   | +0.676        | +0.276   | +0.437  | +0.631        | +0.281   | +0.426  |
| O₄   | -0.356        | -0.330   | -0.327  | -0.310        | -0.298   | -0.295  |
| O₅   | -0.389        | -0.342   | -0.345  | -0.318        | -0.289   | -0.291  |

Furazidine

| Atom | Electrostatic | Mulliken | Natural | Electrostatic | Mulliken | Natural |
|------|---------------|----------|---------|---------------|----------|---------|
| N₁   | -0.663        | -0.731   | -0.699  | -0.617        | -0.728   | -0.695  |
| N₂   | +0.013        | -0.439   | -0.369  | -0.132        | -0.422   | -0.372  |
| O₁   | -0.459        | -0.459   | -0.568  | -0.429        | -0.418   | -0.530  |
| O₂   | -0.451        | -0.433   | -0.533  | -0.401        | -0.372   | -0.474  |
| N₃   | -0.347        | -0.220   | -0.246  | -0.026        | -0.379   | -0.305  |
| O₂   | -0.265        | -0.405   | -0.405  | -0.202        | -0.438   | -0.401  |
| N₄   | +0.681        | +0.275   | +0.437  | +0.653        | +0.279   | +0.427  |
| O₅   | -0.362        | -0.334   | -0.332  | -0.328        | -0.307   | -0.304  |
| O₆   | -0.393        | -0.346   | -0.349  | -0.335        | -0.298   | -0.300  |

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