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A CRITICAL REVIEW ON COMPUTATIONAL STUDY OF ZWITTERIONS IN CEPHAPIRIN TAUTOMERS BY AUSTIN MODEL-1 (AM1) METHOD

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ABSTRACT

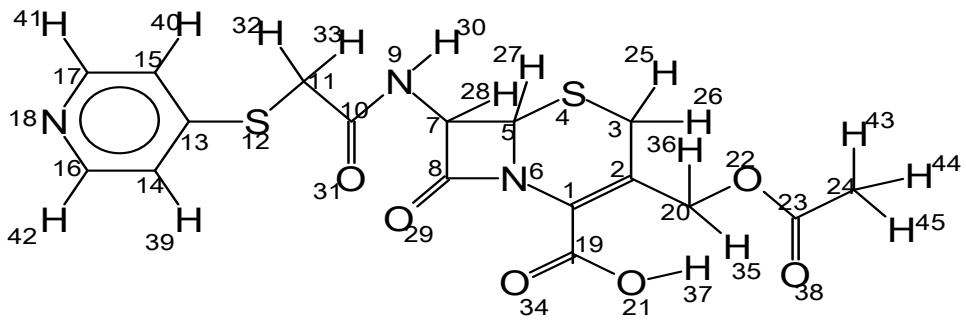
The geometry, conformation and electronic structure of zwitterions in cephapirin tautomers have been reported in the gas phase by semi-empirical molecular orbital AM1 method. In this connection, a critical review on zwitterions of cephapirin tautomers has been discussed in terms of the heats of formation (ΔH_f^o), dipole moment (μ), ionization potential (IP), full atomic charges and energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}).

KEYWORDS: AM1, lactam, lactim, enol, tautomerism, cephapirin, zwitterions, induction effect, frontier molecular orbital.

1. INTRODUCTION

The Cephapirins are β-lactam antibiotics isolated from *Cephalosporium spp*. or prepared semi-synthetically. The cultures of *C. acremonium* inhibited the growth of a wide variety of gram-positive and gram-negative bacteria.[1] The *antibiosis* concept was observed in fungi, which is inhibiting bacterial growth in certain culture media by consuming the bacteria's oxygen and inhibited their growth. i.e. a chemical substance produced by one of the micro-organisms inhibited the growth of the other.[2] These antibiotics contribute a potent and rapid bactericidal action with very low toxic adverse reactions in the host.[3] Cephapirin, which is chemically 3-(acetoxymethyl)-7-[2-(p-pyridylthioacetyl) amino]-8-oxa-5-thia-1-azabycyclo [4.2.0.]oct-2-ene-2-carboxylic acid, which has been recognized active against infections of the upper and lower respiratory tract, skin and related soft tissue, urinary

tract, bones and joints, septicaemias, endocarditic, intra-abdominal and bile tract infections caused by gram(+) organisms.[4] Quantitative structure–activity relationship (QSAR) studies indicate that hydrophobic groups in the side chain appears to be moderately responsible for 45 to 50% protein binding in plasma and cleared rapidly by the kidneys.[5] In practice, the dipolar character of the molecule has been expected to influence selective penetration through the porin channels of the cell membrane[6] and improve oral absorption.[7] Tautomeric equilibrium is predictable for the study of the processes of both organic chemistry and biochemistry[8] and it is reported extensively theoretical and statistical-physical approaches.[9] The stability of tautomers[10] and equilibrium constants in electrostatic reaction field for heterocyclic compounds in aqueous solution[11] was reported. Quantum chemistry is the field in which solutions to the Schrödinger's equation (HΨ = EΨ) are used to predict the properties of molecules and solve chemical problems. It is worthwhile to write a critical review on zwitterions of the tautomers in cephapirin with a view to investigate their polarities, which are an advantage for intrinsic activity against bacterial enzymes involved in cell wall synthesis and cross-linking. This critical review is in continuation of previously reported reviews on computational study of tautomerism in beta-lactam anibiotics[12], protonation of phenethicillin[13] and benzylpenicillin tautomers.[14]



Cephapirin

Figure - 1

2. Importance of Computational method[15]

Austin Model-1 (AM1) is one of the semi-empirical quantum calculation methods, which is based on the neglect of differential diatomic overlap integral approximation, it includes experimental parameters and extensive simplification of the Schrodinger's equation (HΨ=EΨ) to optimize molecules for calculation of various properties to solve chemical

problems.[15] In this way quantum chemistry simulates chemical structure and allows studying chemical phenomena by running calculations on computer rather than by examining reactions experimentally.

Semi-empirical molecular orbital calculations were performed using the AM1 (Austin Model 1) method included in the MOPAC93 in WinMOPACver. 5.13 program by means of Intel P4 PC. Geometry calculations in the ground state (keywords: PRECISE, equivalent to GNORM=5.0, CHARGE, GEO-OK, and MMOK to correct the increase in the barrier to rotation of the amide linkage) were completely optimized until the lowest energy conformation was found. The position of the atom in the molecule is mentioned as subscript as shown in figures-**1**, **2, 3** and **4**. The initial molecular geometry was adopted as Pople's standard data[16], and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne- Prelog terms[17] using s = syn, a = anti, p = peri-planar (0 ± 30^0 & 180 ± 30^0) and all other angles c = clinal.

3. Importance of Austin Model-1 (AM1) method

The optimization of structural parameters (bond lengths and bond angles) of S-2-picolyl-β-N-(2-acetylpyrrole) dithiocarbazate have been calculated by semi-empirical AM1 and PM3 methods[18], compared with those of experimentally available x-ray diffraction data and found in good agreement with experimental values. For bond angles none of the methods produce excellent correlation but out of the two methods AM1 method gives slightly better results than PM3 methods (correlation coefficients, CC=0.506).

AM1 semi-empirical method[19] on isomer structures of four calix[4]resorcinarenes have been functionalized with organic phosphorus groups, but the conformational and configurational structures were realized and optimized by Hyper Chem programmer. The effect of solvents' on the formation ability of ligand has been studied to form metal complexes.[20] M.J.S.Dewar et al[15a] have calculated the effect of solvent on the ability of formation of complexes of acetamide with metals and structural, energetic and electronic characteristics of dimethylacetamide in the state of gas and in solvents by means of the quantum-chemical semi-empirical AM1 method. Semi-empirical calculations of AM1 method[21] has been reported to characterize the self-assembly of carbazole, tetracyanoethylene, 2,3-dichloro-5,6-dicyano-p-benzoquinone, and 2,4,7-trinitro-9-fluorenone. Carbazole is an electron-rich system, while others are electron-deficient systems. Since all of these molecules contain aromatic rings and π systems, their self-organization is mainly based on aromatic interactions

and donor–acceptor interactions. The energetic and physical properties of these molecular systems, such as heat of formation, HOMO–LUMO gap, binding energy, dipole moment, and mean polarizability were calculated. AM1 semi empirical levels are used to calculate the Mullikan's charges and dipole moment of common atoms for twenty six 1, 3-diarylpyrazole derivatives is used as chemometric tool.[22] The model indicates the importance of hydroxyl group at various position of the moiety. The conformational energy surface at compound (Z)-13-hexadecen-11-ynyl acetate as well as the electronic properties of a few analogues were reported at varying the torsion angles by using semi-empirical methods.[23]

AM1 method[24] is applied satisfactorily in many hydrogen-bonded systems especially in pentachlorophenol and dihydroxybenzoquinone-amine systems. The quality of semi-empirical results depends on the nature of the investigated system and its chemical properties which are the targets of the study. The influences of the chemical substitution was studied on the proton transfer process and the strength of the hydrogen bond from the experimental values and correlated with the theoretical values calculated by using AM1 method[25] for the quinones. The proton affinities of the substituted anilines[26] were calculated by using AM1 approach and using it as a basicity scale in the system under investigation. AM1-type semi-empirical quantum chemical calculations[27] are reported to explain differences in herbicidal activity between certain phenoxyacetic acid derivatives. It was found that the proper orientation and shape of the both COOH group and the phenyl moiety mutually and individually affected the observed activities.

AM1 method[28] has been applied successfully to study the chemical and physical properties of metal oxides and different reactions with participation of oxide catalysts. An interesting theoretical study[29] was reported on the Diels-Alder reaction of polychlorinated cyclopentadiene to norbornadiene. They employed semi-empirical AM1methods to study the transition states and energy levels of this, at one time industrially important reaction. They also applied low level ab-initio (HF/3-21) calculations on these AM1 optimized structures to study the transition states of the exo- and endo- approaches of the diene to the dienophile. Energetic of the ground and excited state intra-molecular proton transfer in salicylic acid was reported by using the semi-empirical method AM1[30] at the RHF level as well as with single and pair doubles excitation configuration interaction spanning eight frontier orbitals (PECI). Electron density changes with electronic excitation and tautomerism indicate no zwitterion formation.

The semi-empirical AM1 SCF-MO method[31] was used to study the benzyne mechanism for aromatic nucleophilic substitution of various *m*-substituted chloro-benzenes would induce the formation of 2,3-arynes through their electron-withdrawing resonance or inductive effects. The sites of nucleophilic addition to arynes as predicted here are in fair agreement with expectation and experimental findings. Semi-empirical AM1 SCF-MO calculations[32] was applied to find the structure optimization and conformational inter-conversion pathways of a system containing a six-membered ring. The system has the two symmetrical energy-minimum conformations, chair and twist. The chair conformation has the most stable geometry. Some quantum parameters such as HOMO and LUMO energy, the chemical hardness and chemical potential are discussed.

4. Importance of zwitterions

Amino acids are behaving as amines in some reactions and as carboxylic acids in others are called amphoteric. An internal acid-base reaction may occur in amino acids, due to contain both a positive and a negative charge in the same compound is called a zwitterion. The structure of an amino acid can change with the pH of the solution, i.e. lowering the pH of the solution causes the zwitterion to pick up a proton or increasing the pH of the solution causes the zwitterion to lose a proton. The number of protonated ammonium groups (positive charges) and deprotonated carboxylate groups (negative charges) are equal at a certain pH known as isoelectric point. At the isoelectric point, the pH of the solution affects the charge on the amino acid, to form a zwitterion. Solutions of amino acids and proteins can act as buffers, due to react with both H_3O^+ and OH^- to regulate the pH of blood in the blood proteins. Ultra-filtration technology is an environmentally friendly separation method for the field of biomedicine. Membrane fouling, which results in reduction of separation efficiency, shortening of device life, and increase in cost[33], improvement of the anti-protein-fouling surface has become one of the most important research topics in the field of membrane separation.[34] Anti-protein-fouling materials are typically one of two types: polyethylene glycol or zwitterions. Polyethylene glycol is readily oxidisable in most biochemical solutions, which is greatly reduced the anti-protein adsorption. The antifouling property is not maintained over a long time at high temperature.[35]

Zwitterions have strong hydration capacity by both ion-solvation and hydrogen bonding to form a hydrated layer. The proteins have to destroy the hydration layer by consuming energy to pass the membrane.[36] Charge uniformity not only can maximize the hydration ability of

the zwitterion but also can reduce the electrostatic interaction between membrane surface and protein.[37] Anti-protein fouling of zwitterions is also associated with the uniformity of the distribution of charge and the electrical neutrality. Charge uniformity, close arrangement, small size, and polarity are necessary for anti-protein fouling on the molecular level.[38] In addition, water molecules, zwitterionic structure, and protein will affect the anti-protein-fouling property. With increasing the distance between the positive and negative charges in zwitterions are conducive to enhance the hydration and strengthen the zwitterionic anti-protein resistance.[39]

Typical zwitterions are of the carboxylic acid type, the sulfonic acid type, or the phosphoric acid type. Sulfonic acid and phosphoric acid type zwitterions have been applied to the surface of the polymer membrane in functional modification, and the phospholipid double biomembrane structure[40] showed that similar phospholipids (PC) structure used as the anti-protein adsorption surface. PC zwitterionic monomers are difficult to synthesize, and the single functional group limits their practical application.[41] Thus, sulfonic acid zwitterions had the ability of anti-protein adsorption.[42] The antifouling property was evaluated by static protein adsorption and dynamic ultra filtration of protein solution. Amino acids may exist as zwitterions in solution, the general assumption that zwitterions do not exist in the gas phase has been the subject of recent debate from both experimental and theoretical approaches. Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometry had demonstrated that glycine is unstable as a zwitterion by ~20 kcal/mol.[43] Ab-initio calculations confirm that glycine is unlikely to exist in the gas phase as a zwitterion.[44] However, glycine also has the lowest proton affinity of the amino acids, making it the worst candidate for a gas phase zwitterion. On the other hand, the guanidinium group of arginine (Arg) gives it the highest proton affinity of the amino acids, making Arg the best candidate for a gas phase zwitterion. The first studies on Arg may exist in the zwitterionic form.[45] But experimental[46] and theoretical[47] studies indicate that the isolated Arg monomer is not a zwitterion in the gas phase. On the other hand several recent experiments, supported by theory, suggest that Arg in the presence of a net charge may exist in the zwitterionic state.[48] The recent calculations are indicated the attachment of an electron to glycine may reduce the instability of the glycine zwitterion from 20 to 9 kcal/mol.[49] Compared to other amino acids, Arg possesses a high propensity to form abundant clusters when electro-sprayed into the gas phase.[50] The clustering ability is due to the salt bridges formed by association of the guanidinium group of one Arg with the carboxylate group of another.[51] Zwitterions are critically important in

many biological transformations and are used in numerous chemical processes. The consequences of electrostatic effects on reactivity and physical properties were performed and compared to the experimental electron binding energies. Structures, relative stabilities, and the electron detachment sites also were obtained from the calculations.[52]

At physiological conditions, a majority of biomolecules exist predominantly in the zwitterionic form that usually decides the biological functions. However, zwitterionic amino acids are not geometrically stable in gas phase and this seriously hampers the understanding of their structures, properties and biological functions. The stabilization effects of zwitterionic amino acids of canonical conformers are dependent on water contents, while zwitterionic stability improves monotonously and pronouncedly with increase of water contents.[53-58] Nowadays, continuum models are more popular methods for incorporating solvent effects into theoretical calculations on chemical systems.[59] Gas phase quantum calculations can frequently reproduce the essential features of chemical processes to obtain quantitative and qualitative agreement with experiments.[60] Amino acid chemistry in solution is found in its neutral form in the gas phase, whereas the zwitterionic form dominates in crystalline or aqueous media.[61] *Ab initio* calculations using flexible basis sets show that the zwitterionic form is not an energy minimum *in vacuo.*[62] The interactions with the surroundings must be included into the calculations using continuum models and the analysis of the experimental infrared and Raman spectra of the amino acids in solution.[63] The assignment of the different fundamental vibrations needs accurate calculations on the zwitterionic form with the consideration of the solvent- induced stabilization.

Theoretical works on amino acids chemistry in solution have been restricted to study on glycine zwitterion stability from statistical studies using classical potentials[64] and quantum mechanical studies using the continuum model[65] or small glycine-water clusters.[66] The continuum models are useful tool for interpreting the vibrational spectra of amino acid zwitterions in solution[67] and continuum solvation models[68,69], which are based on the requirements of the system under study. Density functional theory (DFT) methods include correlation energy for obtaining accurate geometrical and thermodynamic data at a fraction of the computational cost of traditional correlated *ab initio* methods.[70] DFT methods on larger systems applied to the study of the gas phase potential energy surface of glycine and alanine.[71] Promising results have also been obtained in the description of proton transfer processes in the hybrid functionals.[72] Density functional methods in combination with the

scaled quantum mechanical (SQM)[73] procedure successfully reproduce the vibrational spectra of a great variety of molecules, for the interpretation and the assignment of the spectra of other systems. The density functional methods give results of good quality to predict and analyze the behaviour of larger amino acids in solution.

5. Importance of induction effect

The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[74] (μ_{ind}) of zwitterions can be estimated with respect to cephapirin by using the equation (1).

$\Delta\mu_{ind}(\text{zwitterion}) = \mu(RH^{\pm}) - \mu(RH)$ . . . (1)

Where $\Delta\mu_{ind}$(zwitterion) is the magnitude of the induction effect, $\mu(RH^{\pm})$ is the dipole moment of zwitterion and μ(RH) is the dipolemoment of cephapirin.

6. Importance of Proton affinity

The position of proton transfer equilibrium can be affected by the nature of the solvent and concentration of the solution. The proton affinity (PA)[75] values for the different nitrogen atoms of cephapirin tautomer (RH) were calculated by using the equation (2).

$PA = \Delta H_f^o(H^+) + \Delta H_f^o(B) - \Delta H_f^o(BH^+)$ . . . (2).

Where PA is the proton affinity, $\Delta H_f^o(B)$ is the heat of formation for the cephapirin, $\Delta H_f^o(BH^+)$ is the heat of formation for protonated cephapirin, and $\Delta H_f^o(H^+)$ is heat of formation for the proton (367.2 kcal/mol). It can be assumed that $\Delta H_f^o(H^+)$ is to be neglected in polar medium, due to the inter- or intra-molecular proton transfer in the equilibrium as per the equation (3).

RH (Polar medium) $\longrightarrow$ $RH^{\pm}$ … (3)

Thus, the equilibrium (2) becomes

$PA = \Delta H_f^o(RH) - \Delta H_f^o(RH^{\pm})$ . . . (4).

Where $\Delta H_f^o(RH)$ is the heat of formation of cephapirin and $\Delta H_f^o(RH^{\pm})$ is the heat of formation of zwitterions. Zwitterions are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium.

7. Computational study on cephapirin tautomerism[76]

The present investigation reveals tautomerism of cephapirin and it may involve either the shifting of hydrogen atom from nitrogen atom in lactam (-HN-C=O) group to the oxygen atom to form lactim (-N=C-O-H) group in the case of **2** or shifting of hydrogen atom from α-carbon atom of β-lactam (-HC-C=O) group to the oxygen atom to form enol (-C=C-O-H) group in the case of **3** or simultaneously shifting of both hydrogen atoms in the case of **4,** as shown in Scheme-1. Tautomeric equilibrium and electronic properties of cephapirin (**1**) in gas phase usually considering isolated molecules which are surrounded by vacuum and it has been evaluated by AM1 method. From the obtained optimized electronic structure of cephapirin tautomers, the mechanism of proton shifting has been studied by comparison of the relative values of net charges at different atoms of the molecule and also observed the predominated tautomers. Taking cephapirin as a neutral molecule (**1**), the molecular geometry and conformations of lactim (**2**), enol (**3**) and lactim-enol (**4**) systems have been determined by full optimization calculations to know the conformational changes in the molecule for the prediction of reactivity and pharmacological action using semi-empirical molecular orbital AM1 method.

7.1. Computational study on electronic structure of cephapirin tautomers

The optimized electronic structure of cephapirin (**1**) and its tautomers (**2** to **4**) are shown in Scheme-1. The calculated heats of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero-atoms of the molecules (**1** to **4**) are presented in Table-I. It is observed that the net charges on N_6-, N_9- and N_{18}- atoms are -0.2357, -0.3626 and -0.1255 respectively in the case of cephapirin (**1**). It is investigated that net charges of nitrogen atoms in the order of $N_{18} < N_6 < N_9$ and at the time of tautomerism more negative charge is observed at N_9- atom in all tautomers of cephapirin.

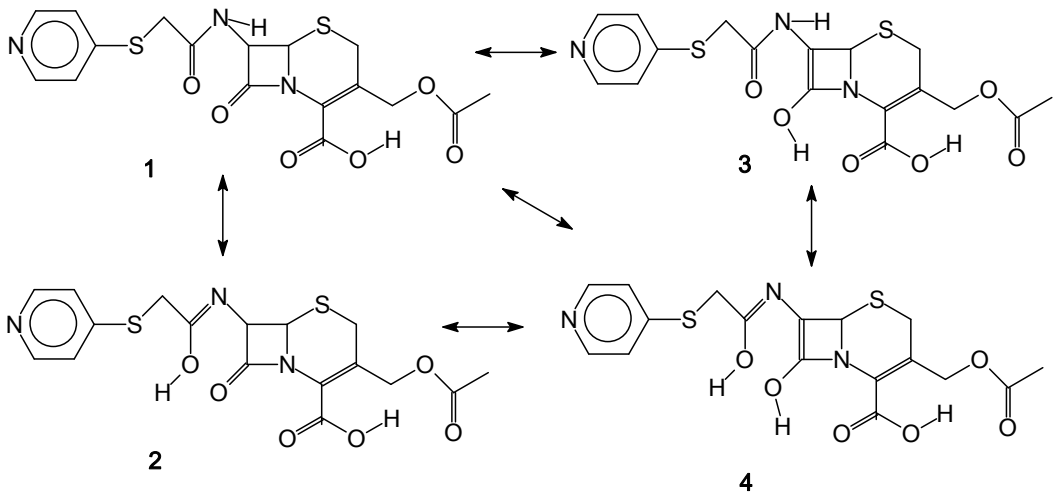
The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** to **4** have more electron-donor character. The results so obtained reveal that the electronic properties and reactivity of molecule depend on its conformational structure. The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules **1** to **4,** due to the presence of same sign.[77] The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of

molecules **3** < **2** < **1** < **4**. Lactim-enol form of cephapirin (**4**) shows higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[74] (μ_{ind}) of molecules can be estimated with respect to cephapirin keto-enol form (**3**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**2**) 0.046D < $\Delta\mu_{ind}$ (**1**) 0.858 D < $\Delta\mu_{ind}$ (**4**) 3.0272 D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **4** < **3** < **2** < **1**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual tautomer. It can be assumed that the electronic properties and reactivity of the tautomer depend on its conformational structure.

Table –I:	**Heat of formation (ΔH_f^o in kcal/mol), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV) and the atomic charges on S_4, S_{12}, N_6, N_9, N_{18}, O_{21}, O_{22}, O_{29}, O_{31}, O_{34} and O_{38} of cephapirin (1), lactim-form (2), Keto-enol form (3) and lactim-enol form (4) from AM1 calculation.**			
Parameters	**1**	**2**	**3**	**4**
ΔH_f^o (kcal/mol)	-139.3971	-132.1969	-113.6674	-107.3375
μ (Debye)	1.6913	0.8793	0.8333	3.8505
E_{HOMO} (eV)	-9.098	-8.836	-8.865	-8.562
E_{LUMO} (eV)	-0.670	-0.650	-0.763	-0.693
Electronic Excitation Energy (eV)	8.428	8.186	8.102	7.869
S_4 (atomic charge)	+0.0706	+0.0977	+0.0945	+0.1304
S_{12} (atomic charge)	+0.2261	+0.2737	+0.2350	+0.2376
N_6 (atomic charge)	-0.2357	-0.2363	-0.1453	-0.1108
N_9 (atomic charge)	-0.3626	-0.2707	-0.2910	-0.1453
N_{18} (atomic charge)	-0.1255	-0.1393	-0.1156	-0.1374
O_{21} (atomic charge)	-0.2796	-0.2829	-0.3144	-0.2831
O_{22} (atomic charge)	-0.2766	-0.2742	-0.2721	-0.2757
O_{29} (atomic charge)	-0.2521	-0.2767	-0.2052	-0.2007
O_{31} (atomic charge)	-0.3341	-0.2850	-0.3242	-0.2825
O_{34} (atomic charge)	-0.3478	-0.3467	-0.3448	-0.3480
O_{38} (atomic charge)	-0.3601	-0.3597	-0.3601	-0.3563

Table –II: Bond lengths of cephapirin (1) and its tautomeric forms (2 to 4) from AM1 calculations.				
Bond lengths (Å)	**1**	**2**	**3**	**4**
C_{10}-N_9	1.3853	1.2949	1.3931	1.3002
O_{31}-C_{10}	1.2434	1.3807	1.2417	1.3861
O_{29}-C_8	1.2184	1.2224	1.3470	1.3484
C_8-N_6	1.4303	- -	1.4635	1.4490
H-O_{31}	- -	1.4247	- -	0.9714
H-O_{29}	- -	0.9717	0.9774	0.9745

Equilibrium is normally established in polar solvents, in order to investigate the stable tautomer and it is found out the shifts of protons of cephapirin (**1**). The stable tautomers of cephapirin (1) are confirmed by the calculated heats of formation with full geometry optimization. The tautomers can exist in ***anti***- or ***syn***-conformations. Its conformation can be assigned by comparison of its geometry and electronic structure as per Scheme-1. Three tautomeric forms of cephapirin (**1**) are possible, in the great majority of cases the molecules at chemical equilibrium under ordinary conditions. Instances are known when tautomeric forms are stable under ordinary conditions which are capable of inter-conversion at higher temperatures, often with the aid of catalyst. Fully optimized AM1 calculations scrutinize only the main data of bond lengths (Table-II) and dihedral angles (Table-IV) of tautomers (**2** to **4**) for the sake of simplicity. All tautomers are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium. As per electron excitation energies (ΔE) (in eV), it is observed the reactivity is decreased in the order of **4** > **3** > **2** > **1**. It is confirmed that cephapirin (**1**) is more stable than its tautomers. The shifting of H_{30}-proton and H_{28}-proton of cephapirin **(1)** to respective O_{31}-atom and O_{29}-atom are predicted for the formation of respective lactim-form(**2**) and enol-form (**3**). The simultaneous shifting of H_{30}-proton and H_{28}-proton of cephapirin **(1)** to respective O_{31}- atom and O_{29}- atom is predicted for the formation of lactim-enol form **(4)** of cephapirin.



1 to 2 & 3 to 4 - Lactam-lactim tautomerism
1 to 3 & 2 to 4 - Keto-enol tautomerism
1 to 4 - Lactam-lactim & Keto-enol tautomerism

Scheme - 1

The AM1 calculated heat of formation, and the tautomeric equilibrium constants $logK_T$ was calculated[78] according to the equation (5):

$$logK_T = \frac{\Delta G_T}{2.303\ R\ T} \approx \frac{\delta\Delta H_f^o}{2.303\ R\ T} \quad \text{- - - (5)}$$

Where ΔG_T is the free energy of the tautomeric equilibrium, $\delta\Delta H_f^o$ is the difference in the calculated heats of formation of tautomeric species participating in this equilibrium. R is the gas constant and T is the absolute temperature. From this equation (1), $logK_T$ -values and the change of net charges were calculated and incorporated in Table- III. It is observed that the tautomeric equilibrium is increased in the order of $logK_{T4} < logK_{T1} < logK_{T3} < logK_{T2} < logK_{T5}$. At the time of tautomeric conversion of (**1**) to (**2**), (**1**) to ((**4**) and (**3**) to (**4**) the net charges are increased at N_{18}- atom and decreased at all other hetero atoms. In the case of tautomeric conversion of (**1**) to (**3**) the net charge is increased at O_{21}- atom and decreased at all other hetero-atoms, but the tautomeric conversion of (**2**) to (**4**), the net charge is increased at O_{34}-atom and decreased at all other hetero-atoms.

Table-III	**Tautomeric Equilibrium of cephapirin.**			
$logK_T$	**Equilibrium**	**$logK_T$ - Values**	**Change of Net Charges on Hetero-atoms**	
			Increasing	**Decreasing**
$logK_{T1}$	**1 ↔ 2**	5.28	N_{18}, O_{21}, O_{29}	N_9, O_{22}, O_{31}
$logK_{T2}$	**1 ↔ 3**	18.86	O_{21}	N_6, N_9, N_{18}, O_{22}, O_{29} O_{31}
$logK_{T3}$	**2 ↔ 4**	18.22	O_{34}	N_6, N_9, O_{29}
$logK_{T4}$	**3 ↔ 4**	4.64	N_{18}, O_{34}	N_6, N_9, O_{21}, O_{29}, O_{31}, O_{38}
$logK_{T5}$	**1 ↔ 4**	23.49	N_{18}, O_{21}	N_6, N_9, O_{22}, O_{29}, O_{31}, O_{38}

From the Table-II and Scheme-1, it is observed that cephapirin (**1**) would undergo lactam-lactim tautomerism and form lactim of cephapirin (**2**) with increasing bond length of O_{31}-C_{10} (1.3807 Å) and decreasing bond length of C_{10}-N_9 (1.2949 Å) with the formation of H-O_{31} bond (0.9717Å). It is confirmed that cephapirin (**1**) may undergo lactam-enol tautomerism, and form cephapirin enol (**3**) with increasing bond length of O_{29}-C_8 (1.3470 Å) and C_8-N_6 (1.4635 Å) with the formation of H-O_{29} bond (0.9774 Å). But the formation of lactim-enol tautomerism (**4**) from cephapirin (**1**) is observed with increasing bond lengths of O_{31}-C_{10} (1.3861 Å) and O_{29}-C_8 (1.3484 Å) and C_8-N_6 (1.4490 Å), decreasing bond length of C_{10}-N_9 (1.3002 Å) with the formation of H-O_{31} bond (0.9714Å) and H-O_{29} bond (0.9745 Å).

7.2. Computational study on conformations of cephapirin tautomers

The spatial arrangement of atoms in tautomers are considered to study the conformations of cephapirin (**1**), and its lactim form (**2**), enol form (3) and lactim-enol form (**4**) of cephapirin with a view to investigate molecular deformations. These can exist in *anti*- or *syn*-conformation, according to the position of atoms. In this context, the change in energy content of the protonation may depend on the changes in the parameters of dihedral angles. Fully optimized AM1 calculations for the sake of simplicity, scrutinize only the main data of dihedral angles (Table-IV) of molecules (**1** to **4**).

As per Scheme-1, the H_{30}-proton shifting from N_9- atom to O_{31}- atom in the cephapirin **(1)** is predicted for the formation of lactim-form (**2**). The conformations of ***–sc*** of $C_{10}N_9C_7C_5$ and $C_{15}C_{13}S_{12}C_{11}$ are changed to conformation ***-ap***. The conformation of +***ap*** of $C_{19}C_1C_2C_3$ and $C_{24}C_{23}C_{22}C_{20}$ are changed to *–ap* conformation. The ***–sc*** of $C_{13}S_{12}C_{11}C_{10}$ and ***+ac*** of $O_{22}C_{20}C_2C_3$ conformations are changed to ***+sc*** conformation. Dihedral angle of $C_{14}C_{13}S_{12}C_{11}$, $C_{23}O_{22}C_{20}C_2$ and $O_{31}C_{10}N_9C_7$ are changed to respectively from +***ac*** to ***+sp, -sc*** to ***–ac*** and ***+sp*** to ***-sp*** conformations and all other conformations are moderately changed. At the time of lactam-lactim rearrangement, the lactim-form of cephapirin (**2**) is formed with the ***+sp*** conformation in the case of dihedral angle of $HO_{31}C_{10}N_9$. If the H_{28}-proton shifting from C_7-atom to O_{29}- atom in the cephapirin **(1)** is predicted in the formation of enol-form(**3**). The change of conformation from +***ap*** of $C_{24}C_{23}O_{22}C_{20}$ and $H_{37}C_{21}C_{19}C_1$ are changed to ***-ap*** conformation. The conformation of ***–sc*** of $C_{10}N_9C_7C_5$ and $C_{15}C_{13}S_{12}C_{11}$ are changed to ***–ac*** conformation. Dihedral angle of ***–sc*** of $C_{13}S_{12}C_{11}C_{10}$ and ***+ac*** of $C_{14}C_{13}S_{12}C_{11}$, are changed to ***+sc*** conformation. Dihedral angel of $O_{31}C_{10}N_9C_7$ and $C_{11}C_{10}N_9C_7$ are changed respectively from ***+sp*** to ***–sp*** and ***–ap*** to ***+ap*** conformations. At the time of keto-enol rearrangement, the enol-form of cephapirin (**3**) is formed with the ***+sc*** conformation in the case of dihedral angle of $HO_{29}C_8N_6$.

Table - IV	Dihedral angle (^{0}) of cephapirin (1), lactim-form (2), Keto-enol form (3) and lactim-enol form (4) from AM1 calculation.							
Dihedral angle (^{0})	1		2		3		4	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$O_{31}C_{10}N_9C_7$	+2.16	***+sp***	-1.59	***-sp***	-2.03	***-sp***	-2.97	***-sp***
$C_{10}N_9C_7C_5$	-35.44	***-sc***	-150.86	***-ap***	-128.30	***-ac***	-15.27	***-sp***
$C_{11}C_{10}N_9C_7$	-176.78	***-ap***	-179.23	***-ap***	+179.81	***+ap***	+179.65	***+sp***
$S_{12}C_{11}C_{10}N_9$	-32.88	***-sc***	-84.25	***-sc***	-69.91	***-sc***	-125.77	***-ac***
$C_{13}S_{12}C_{11}C_{10}$	-79.79	***-sc***	+78.83	***+sc***	+86.54	***+sc***	+76.22	***+sc***
$C_{14}C_{13}S_{12}C_{11}$	+139.89	***+ac***	+24.79	***+sp***	+69.19	***+sc***	+11.24	***+sp***
$C_{15}C_{13}S_{12}C_{11}$	-45.08	***-sc***	-160.13	***-ap***	-117.14	***-ac***	-171.74	***-ap***
$C_{19}C_1C_2C_3$	+178.43	***+ap***	-179.21	***-ap***	+172.04	***+ap***	+179.51	***+ap***
$C_{20}C_2C_3C_1$	-178.07	***-ap***	-178.22	***-ap***	-179.20	***-ap***	+179.35	***+ap***
$O_{22}C_{20}C_2C_3$	+104.19	***+ac***	+74.85	***+sc***	+110.12	***+ac***	+61.12	***+sc***
$C_{23}O_{22}C_{20}C_2$	-82.31	***-sc***	-91.85	***-ac***	-80.60	***-sc***	-98.26	***-ac***
$C_{24}C_{23}O_{22}C_{20}$	+179.81	***+ap***	-175.17	***-ap***	+179.45	***-ap***	-176.59	***-ap***
$H_{37}O_{21}C_{19}C_1$	+178.53	***+ap***	+178.15	***+ap***	-179.82	***-ap***	+178.08	***+ap***
$HO_{31}C_{10}N_9$	-	-	+11.59	***+sp***	- -	-	+156.77	***+ap***
$HO_{29}C_8N_6$	-	-	- -	- -	+44.12	***+sc***	+172.25	***+ap***
*Conformational analyses using prefixes a = anti, s = syn, p = periplanar, c = clinal, and = & - signs.[17]								

The simultaneous shifting of H_{30}-proton from N_9- atom and H_{28}-proton from C_7-atom of cephapirin **(1)** to respective O_{31}- atom and O_{29}- atom is predicted for the formation of lactim-enol form **(4)**. The conformations of ***–ap*** of $C_{11}C_{10}N_9C_7$ and ***+ac*** of $C_{14}C_{13}S_{12}C_{11}$ are changed to ***+sp*** conformation, the conformations of ***–sc*** of $C_{13}S_{12}C_{11}C_{10}$ and +ac of $O_{22}C_{20}C_2C_3$ are changed to ***+sc*** conformation and the conformations of ***–sc*** of $S_{12}C_{11}C_{10}N_9$ and $C_{23}O_{22}C_{20}C_2$ are changed to ***–ac*** conformation to form stable conformation and rest of positions have moderate changes. Dihedral angle of $C_{20}C_2C_3C_1$, $O_{31}C_{10}N_9C_7$, $C_{15}C_{13}S_{12}C_{11}$ and $C_{24}C_{23}O_{22}C_{20}$ are changed respectively ***-ap*** to ***+ap***, ***+sp*** to ***–sp***, ***-sc*** to ***–ap*** and ***+ap*** to ***-ap*** conformation and all other conformations are moderately changed. It is also observed that the shifting of H_{30}-proton from N_9- atom and H_{28}-proton from C_7-atom of cephapirin **(1)** to respective O_{31}- atom and O_{29}- atom is predicted for the formation of lactim-enol form **(4)** with the formation of ***+ap*** conformation in the case of $HO_{31}C_{10}N_9$ and $HO_{29}C_8N_6$ dihedral angles.

8. Computational study on cephapirin zwitterions[79]

It has attracted much attention that cephapirin exists as zwitterions over a broad pH range and is considerably increased polarity; the present investigation focuses on the evaluation of the significance of the molecular conformation and electronic properties of cephapirin (**2**), its anion (**1**) and zwitterions $RH^{\pm}$ (**3**, **4** & **5**). The mechanism of proton transfer in cephapirin has

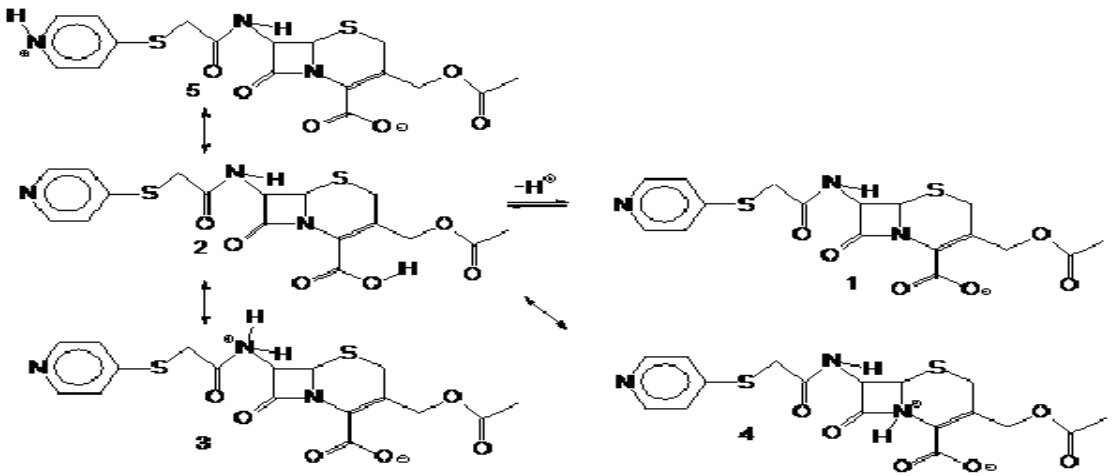
been studied by the different positions of net charges on nitrogen atoms in the molecule. Taking cephapirin, as a neutral molecule (RH) (**2**), the conformation and electronic structures of zwitterions $RH^{\pm}$ (**3**,**4** & **5**) system, in which are included $RH^{\pm}$ ($N_9H^{\pm}$) (**3**), $RH^{\pm}$ ($N_6H^{\pm}$) (**4**) and $RH^{\pm}$ ($N_{18}H^{\pm}$) (**5**) have been determined by full optimization calculations, using semi-empirical molecular orbital AM1 method as per Scheme-2.

8.1. Computational study on electronic structure of cephapirin zwitterions

The optimized electronic structure of cephapirin (**2**), anion (**1**), and its zwitterions $RH^{\pm}$ (**3, 4** and **5**) along with the numbering of the system in this context are shown in Figure -1. The calculated heats of formation (ΔH_f^o), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **5**) are presented in Table-V. The net charges on N_6-, N_9- and N_{18}- atoms are -0.2357, -0.3626 and -0.1255 respectively in the case of neutral molecule cephapirin (2). Usually, the nitrogen atom with larger negative value of net charge accepts proton more easily. Thus, the sequence of protonation for nitrogen atoms in cephapirin is increasing in the order of $N_{18} < N_6 < N_9$. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed due to the presence of same sign.[77]

The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of molecules **2** < **4** < **5** < **1** < **3**. Zwitterion $RH^{\pm}$ ($N_9H^{\pm}$) (**3**) shows higher dipole moment. The electronegative heteroatoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[74] (μ_{ind}) of zwitterions can be estimated with respect to neutral molecule cephapirin (**2**) by using the equation (1) and found in the order of $\Delta\mu_{ind}$(**4**) 2.5460D < $\Delta\mu_{ind}$(**5**) 5.2691D < $\Delta\mu_{ind}$(**3**) 18.2268D. The results so obtained reveal that the electronic properties and reactivity of the molecule depend on its conformational structure. From the reactivity point of view, the search of protonation sites of cephapirin molecule having different positions of oxygen and nitrogen atoms is important. According to the heat of formation (ΔH_f^o) data, the stability of the compounds have decreased in the order of **1** < **2** < **5** < **4** < **3**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual ions or molecules.

Zwitterions are formed with the difference in the heat of formation (ΔH_f^o) of +88.0208 kcal/mol, +81.7465 kcal/ mol and +19.1816 kcal/ mol respectively in the conversion of (**2**) to (**3**), (**2**) to (**4**) and (**2**) to (**5**). It can be predicted that the conversion of neutral molecule cephapirin (**2**) to zwitterion (**5**) is lower energy process than the conversion of (**2**) to (**3**) and (**2**) to (**4**). The protonation site of cephapirin (**2**), containing N_9- atom is predicted to be the main basic centre of molecule (**2**). However, negative atomic charges are also present on the other atoms of the molecule. The proton shifting from O_{21}- atom to N_9- atom in the case of neutral molecule cephapirin (**2**) to zwitterion (**3**) is considered by increasing net atomic charges at S_{12}-, O_{21}-, O_{34}- and O_{38}- and decreasing at S_4-, N_6-, N_9-, N_{18}-, O_{22}-, O_{29}- and O_{31}- atoms. The proton shifting from O_{21}- atom to N_6- atom in the case of (**2**) to (**4**) is considered by decreasing net atomic charges at N_9-, O_{21}-, O_{22}- and O_{34}- and increasing at S_4-, S_{12}-, N_6-, N_{18}-, O_{29}-, O_{31}- and O_{38}- atoms. When, the proton transfer from O_{21}- atom to N_{18}- atom in the case of (**2**) to (**5**) is considered by decreasing net atomic charges at S_{12}-, O_{22}-, O_{29}-, O_{31}- and O_{34}- and increasing at S_4-, N_6-, N_9-, N_{18}-, O_{21}- and O_{38}- atoms.



Scheme - 2

Equilibrium is normally established in polar solvents by rapid inter- or intra-molecular proton transfer from O_{21}-atom to N_9-, N_6- and N_{18}- atoms of cephapirin as shown in Scheme-2. When one zwitterion is formed predominantly in a polar solution, its conformation can be assigned by comparison of its geometry and electronic structure. The position of proton transfer equilibrium can be affected by the nature of the solvent and concentration of the solution. The proton affinity (PA)[75] values for the different nitrogen atoms of cephapirin neutral molecule RH (**2**) were calculated by using the equation (4). The proton affinity is in the order of N_9 (-88.0208 kcal/mol) > N_6 (-81.7465 kcal/mol) > N_{18} (-19.1816 kcal/mol).

However, zwitterion (**3**) appears to be more stable. Cephapirin[6] is unstable in aqueous solutions and it is often directed to keep injectable preparations. The pKa of cephapirin is HA = 2.67 and HB^+ = 4.49. Zwitterions are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium.

Table –V	**Heat of formation (ΔH_f^o in kcal/mol), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV) and the atomic charges on S_4, S_{12}, N_6, N_9, N_{18}, O_{21}, O_{22}, O_{29}, O_{31}, O_{34} and O_{38} of Cephapirin (2), anion (1) and its zwitterions (3, 4 & 5) from AM1 calculation.**				
Parameters	**R^-(1)**	**RH (2)**	**$RH^{\pm}$(3)**	**$RH^{\pm}$(4)**	**$RH^{\pm}$(5)**
ΔH_f^o (kcal/mol)	-167.6001	-139.3971	-51.3763	-57.6506	-120.2155
μ (Debye)	19.3772	1.6913	19.9181	4.5460	6.9604
E_{HOMO} (eV)	-5.259	-9.098	-8.332	-9.076	-9.301
E_{LUMO} (eV)	+0.938	-0.670	-2.534	-0.577	-1.026
S_4 (atomic charge)	-0.0224	+0.0706	+0.0589	+0.2572	+0.0994
S_{12} (atomic charge)	+0.1969	+0.2261	+0.3315	+0.2625	+0.2173
N_6 (atomic charge)	-0.2242	-0.2357	-0.2032	-0.3929	-0.2701
N_9 (atomic charge)	-0.3492	-0.3626	-0.0883	-0.3572	-0.3723
N_{18} (atomic charge)	-0.1293	-0.1255	-0.1041	-0.1392	-0.1591
O_{21} (atomic charge)	-0.5399	-0.2796	-0.5516	-0.2516	-0.3038
O_{22} (atomic charge)	-0.2637	-0.2766	-0.2672	-0.2700	-0.2674
O_{29} (atomic charge)	-0.2720	-0.2521	-0.2165	-0.2837	-0.2260
O_{31} (atomic charge)	-0.3415	-0.3341	-0.1601	-0.3363	-0.3142
O_{34} (atomic charge)	-0.5109	-0.3478	-0.4836	-0.2334	-0.2987
O_{38} (atomic charge)	-0.3925	-0.3601	-0.3816	-0.3978	-0.3635

8.2, Computational study on conformations of cephapirin zwitterions

The spatial arrangement of atoms in a molecule is considered to study the conformations of cephapirin (**2**), anion (**1**) and zwitterions (**3**, **4** & **5**) with a view to undergoing molecular deformations. Zwitterions can exist in *anti*- or *syn*- conformation, according to the position of atoms. In this context, the change in energy content of the zwitterion may depend on the changes in the parameters of dihedral angles. The conformational analyses of zwitterions reveal about molecular deformations. Figure-1 illustrates the atomic numbering of cephapirin (**2**). Fully optimized AM1 calculations scrutinize only the main data of dihedral angles (Table-VI) of molecules (**1** to **5**) for the sake of simplicity. It has been found that the length of N_{18}-H bond (1.7914 A^0) in $RH^{\pm}$ ($N_{18}H^{\pm}$) (**5**) system is larger than that of N_9-H bond (1.0356 A^0) in $RH^{\pm}$ ($N_9H^{\pm}$) (**3**) and N_6-H bond (0.9866 A^0) in $RH^{\pm}$ ($N_6H^{\pm}$) (**4**) system. From the study it can be concluded that the sequence of proton transfer for nitrogen atoms in cephapirin (**2**), agrees well with the results attained. The stable conformations of zwitterions (**3**, **4** and **5**) are forming with the proton transfer to alternate nitrogen atom of the molecule.

From the Table-VI and Scheme-2, it can be concluded that the anion R^- (**1**) is formed with the removal of a proton on O_{21}- atom of RH (**2**), and the change of conformation from +*sp* of $H_{30}N_9C_7C_5$ and –*ap* of $C_{20}C_2C_3C_1$ are changed to +*ap*. Dihedral angle of $C_{10}N_9C_7C_5$ and $O_{22}C_{20}C_2C_3$ are changed with conformations, -*sc* to –*sp* and +*ac* to +*sc* respectively to form stable anion and rest of positions have moderate changes. The zwitterion $RH^{\pm}$ ($N_9H^{\pm}$) (**3**) is formed by the proton transfer between O_{21}-atom to N_9-atom of RH (**2**) with the change of conformation from -*sc* of $C_{10}N_9C_7C_5$, -*ap* of $C_{11}C_{10}N_9C_7$ and –*ap* of $C_{20}C_2C_3C_1$ are changed to +*ap* conformation. Dihedral angle of +*ap* of $C_{19}C_1C_2C_3$ and $C_{24}C_{23}O_{22}C_{20}$ are changed to -*ap* conformation. The conformations are changed from +*sp* to +*sc*, -*sc* to +*sc*, +*ac* to +*sc*, -*sc* to –*ac*, +*ac* to +*sc*, -*sc* to -*ac* and +*sp to* -*sp* in case of $H_{30}N_9C_7C_5$, $C_{13}S_{12}C_{11}C_{10}$, $C_{14}C_{13}C_{12}C_{11}$, $C_{15}C_{13}S_{12}C_{11}$, $O_{22}C_{20}C_2C_3$, $C_{23}O_{22}C_{20}C_2$ and $O_{31}C_{10}N_9C_7$ respectively to form more stable zwitterion and all other positions are not as much of altered.

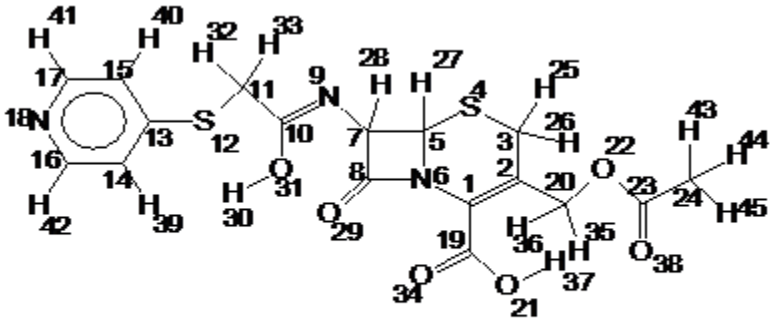
Table – VI:	**Dihedral angle (^{0}) of Cephapirin (2), anion (1) and Cephapirin zwitterions (3, 4, 5) from AM1 calculation.**									
Dihedral angle (^{0})	**R^-(1)**		**RH (2)**		**$RH^{\pm}$(3)**		**$RH^{\pm}$(4)**		**$RH^{\pm}$(5)**	
	Angle	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**
$H_{30}N_9C_7C_5$	+166.44	+*ap*	+2.16	+*sp*	+54.64	+*sc*	-62.29	-*sc*	+6.51	+*sp*
$N_9C_7C_5S_4$	-122.68	-*ac*	-120.79	-*ac*	-139.59	-*ac*	-120.38	-*ac*	-131.06	-*ac*
$C_{10}N_9C_7C_5$	-19.68	-*sp*	-35.44	-*sc*	+174.90	+*ap*	+114.99	+*ac*	-157.53	-*ap*
$C_{11}C_{10}N_9C_7$	-174.11	-*ap*	-176.78	-*ap*	+153.91	+*ap*	+177.57	+*ap*	+177.66	+*ap*
$S_{12}C_{11}C_{10}N_9$	-40.75	-*sc*	-32.88	-*sc*	-75.16	-*sc*	+54.73	+*sc*	-100.42	-*ac*
$C_{13}S_{12}C_{11}C_{10}$	-80.46	-*sc*	-79.79	-*sc*	+84.62	+*sc*	+75.14	+*sc*	+25.72	+*sp*
$C_{14}C_{13}S_{12}C_{11}$	+127.29	+*ac*	+139.89	+*ac*	+72.95	+*sc*	+172.68	+*ap*	+90.89	+*ac*
$C_{15}C_{13}S_{12}C_{11}$	-57.59	-*sc*	-45.08	-*sc*	-113.92	-*ac*	-7.83	-*sp*	-89.51	-*sc*
$C_{19}C_1C_2C_3$	+179.94	+*ap*	+178.43	+*ap*	-176.18	-*ap*	+179.55	+*ap*	+173.01	+*ap*
$C_{20}C_2C_3C_1$	+178.17	+*ap*	-178.07	-*ap*	+179.93	+*ap*	-178.18	-*ap*	+177.33	+*ap*
$O_{21}C_{19}C_1C_2$	+115.00	+*ac*	+122.19	+*ac*	+134.90	+*ac*	+140.47	+*ac*	+112.79	+*ac*
$O_{22}C_{20}C_2C_3$	+75.35	+*sc*	+104.19	+*ac*	+67.70	+*sc*	+113.97	+*ac*	+125.15	+*ac*
$C_{23}O_{22}C_{20}C_2$	-87.83	-*sc*	-82.31	-*sc*	-90.60	-*ac*	-63.88	-*sc*	-81.67	-*sc*
$C_{24}C_{23}O_{22}C_{20}$	+176.43	+*ap*	+179.81	+*ap*	-174.63	-*ap*	-178.61	-*ap*	-176.09	-*ap*
$O_{31}C_{10}N_9C_7$	+6.30	+*sp*	+2.16	+*sp*	-24.22	-*sp*	-1.49	-*sp*	-7.11	-*sp*
$O_{34}C_{19}C_1C_2$	-63.03	-*sc*	-57.28	-*sc*	-45.41	-*sc*	-39.29	-*sc*	-62.26	-*sc*
$H_{37}O_{21}C_{19}C_1$	-	-	+178.53	+*ap*	-	-	-	-	-	-
$HN_9C_7C_5$	-	-	-	-	-62.21	-*sc*	-	-	-	-
$HN_6C_5S_4$	-	-	-	-	-	-	+53.53	+*sc*	-	-
$HN_{18}C_{17}C_{15}$	-	-	-	-	-	-	-	-	-155.49	-*ap*
* Conformational analyses using prefixes a = anti, s = syn, p = periplanar, c=clinal, and + & - signs.[17]										

If the transfer of proton between O_{21}-atom to N_6-atom of RH (**2**) forms the zwitterion $RH^{\pm}$ ($N_6H^{\pm}$) (**4**), with the change of dihedral angle of $S_{12}C_{11}C_{10}N_9$ and $C_{13}S_{12}C_{11}C_{10}$ of –*sc* to +*sc*

conformation. Dihedral angle of $C_{11}C_{10}N_9C_7$ of *–ap* and $C_{14}C_{13}C_{12}C_{11}$ of *–sc* are changed to *+ap* conformation. Dihedral angle of $H_{30}N_9C_7C_5$, $C_{10}N_9C_7C_5$, $C_{15}C_{13}C_{12}C_{11}$, $C_{24}C_{23}O_{22}C_{20}$ and $O_{31}C_{10}N_9C_7$ are changed with conformations, *-sp* to *–sc*, *–sc* to *+ac*, *-sc* to *–sp*, *+ap* to *-ap* and *+sp to –sp* respectively to form stable zwitterion and all other positions are altered insignificant. If the transfer of proton between O_{21}-atom to N_{18}-atom of RH (**2**) forms the zwitterion $RH^{\pm}$ ($N_{18}H^{\pm}$) (**5**), with the change of dihedral angle of *-sc* of $C_{10}N_9C_7C_5$ and *+ap* of $C_{24}C_{23}O_{22}C_{20}$ to *–ap* conformation. The conformations from *–ap* of $C_{11}C_{10}N_9C_7$ and $C_{20}C_2C_3C_1$ are changed to *+ap* conformation. The change of conformations from *-sc* of $S_{12}C_{11}C_{10}N_9$, *-sc* of $C_{13}S_{12}C_{11}C_{10}$ and *+sp* of $O_{31}C_{10}N_9C_7$ are converted respectively to *-ac*, *+sp* and *-sp* conformations and rest of positions have moderate changes.

9. Computational study on zwitterions in cephapirin-lactim tautomer[80]

The molecular conformation and electronic properties of cephapirin (1) and its enol form (2), zwitterions $RH^{\pm}$ (3, 4 & 5) and anion (6) have been evaluated. The mechanism of proton transfer in cephapirin (1) to lactim-form (2) through lactam-lactim tautomerism has been studied. Taking cephapirin-lactim form, as a neutral molecule (RH) (2), the conformation and electronic structures of zwitterions $RH^{\pm}$ (3,4 & 5) system, in which are included $RH^{\pm}$ ($N_9H^{\pm}$) (3), $RH^{\pm}$ ($N_6H^{\pm}$) (4) and $RH^{\pm}$ ($N_{18}H^{\pm}$) (5) have been determined by full optimization calculations, using semi-empirical molecular orbital AM1 method.



Cephapirin-lactim form

Figure - 2

9.1. Computational study on electronic structure of cephapirin-lactim zwitterions

The optimized electronic structure of cephapirin (**1**) and its lactim form (**2**), zwitterions $RH^{\pm}$ (**3, 4** and **5**) and anion (**6**) are shown in Scheme-3. In this context, the numbering of lactim-form (2) is shown in Figure -2. The calculated heats of formation (ΔH_f°), dipole moment (μ),

the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **6**) are presented in Table-VII. The net charges on N_6-, N_9- and N_{18}-atoms are -0.2363, -0.2707 and - 0.1393 respectively in the case of neutral cephapirin-lactim (2). Usually, the nitrogen atom with larger negative value of net charge accepts proton more easily. Thus, the sequence of protonation for nitrogen atoms in cephapirin enol is increasing in the order of $N_{18} < N_6 < N_9$. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed due to the presence of same sign.[77]

Table –VII:	**Heat of formation (ΔH^{o} in kcal/mol), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV) and the atomic charges on S_4, S_{12}, N_6, N_9, N_{18}, O_{21}, O_{22}, O_{29}, O_{31}, O_{34} and O_{38} of cephapirin (1), lactim-form of cephapirin (2), its zwitterions (3, 4 & 5) and anion (6) from AM1 calculation.**					
Parameters	**RH (1)**	**RH (2)**	**$RH^{\pm}$ (3)**	**$RH^{\pm}$ (4)**	**$RH^{\pm}$ (5)**	**R^{-}(6)**
ΔH_f^{o} (kcal/mol)	-139.3971	-132.1969	-68.6118	-22.6980	-88.5084	-164.6227
μ (Debye)	1.6913	0.8793	18.1518	6.4816	11.2453	11.6843
E_{HOMO} (eV)	-9.098	-8.836	-8.349	-9.328	-8.521	-5.614
E_{LUMO} (eV)	-0.670	-0.650	-2.844	-2.349	-2.233	+1.810
S_4 (atomic charge)	+0.0706	+0.0977	+0.0503	+0.0604	+0.0691	+0.0023
S_{12} (atomic charge)	+0.2261	+0.2737	+0.3289	+0.1943	+0.2719	+0.1803
N_6 (atomic charge)	-0.2357	-0.2363	-0.1977	-0.4069	-0.2532	-0.2210
N_9 (atomic charge)	-0.3626	-0.2707	-0.2203	-0.2220	-0.2765	-0.2470
N_{18} (atomic charge)	-0.1255	-0.1393	-0.1117	-0.1156	-0.0466	-0.1287
O_{21} (atomic charge)	-0.2796	-0.2829	-0.5554	-0.3682	-0.6431	-0.5322
O_{22} (atomic charge)	-0.2766	-0.2742	-0.2706	-0.2644	-0.2502	-0.2658
O_{29} (atomic charge)	-0.2521	-0.2767	-0.2899	-0.2720	-0.2681	-0.3021
O_{31} (atomic charge)	-0.3341	-0.2850	-0.1901	-0.2858	-0.2777	-0.3011
O_{34} (atomic charge)	-0.3478	-0.3467	-0.4834	-0.3496	-0.4309	-0.5269
O_{38} (atomic charge)	-0.3601	-0.3597	-0.3746	-0.3496	-0.4478	-0.3876

The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of molecules **2** < **1** < **4** < **5** < **6** < **3**. Zwitterion $RH^{\pm}$ ($N_9H^{\pm}$) (**3**) shows higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[74] (μ_{ind}) of zwitterions can be estimated with respect to neutral molecule cephapirin-lactim (**2**) by using the equation (1) and observed in the order of $\Delta\mu_{ind}$(**4**) 5.6023D < $\Delta\mu_{ind}$(**5**) 10.366D < $\Delta\mu_{ind}$(**3**) 17.2725D. The results so obtained reveal that the electronic properties and reactivity of the molecule depend on its conformational structure. From the reactivity point of view, the search of protonation sites of cephapirin-lactim (**2**) having different positions of oxygen and nitrogen

atoms are important. According to the heat of formation (ΔH_f ^{o}) data, the stability of the compounds have decreased in the order of **6** < **1** < **2** < **5** < **3** < **4**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual ions or molecules. Cephapirin (**1**) is stable than lactim form (**2**) according to the heat of formation (ΔH_f^o) data. But lactam-lactim tautomeric forms are depending upon the polarity of solutions.[81] Zwitterions are formed with the difference in the heat of formation of +63.5851 kcal/mol, +109.4989 kcal/ mol and +43.6885 kcal/ mol respectively in the conversion of (**2**) to (**3**), (**2**) to (**4**) and (**2**) to (**5**). It can be predicted that the conversion of cephapirin-lactim (**2**) to zwitterion (**5**) is lower energy process than the conversion of (**2**) to (**3**) and (**2**) to (**4**). The protonation site of cephapirin-lactim (**2**), containing N_9- atom is predicted to be the main basic centre of molecule (**2**). However, negative atomic charges are also present on the other atoms of the molecule. The proton shifting from O_{21}- atom to N_9- atom in the case of neutral cephapirin-lactim (**2**) to zwitterion(**3**) is considered by increasing net atomic charges at S_{12}-, O_{21}-, O_{29}-, O_{34}- and O_{38}- and decreasing at S_4-, N_6-, N_9-, N_{18}-, O_{22}-, and O_{31}- atoms. The proton shifting from O_{21}- atom to N_6- atom in the case of (**2**) to (**4**) is considered by decreasing net atomic charges at S_4-, S_{12}-, N_9-, N_{18}-, O_{22}-, O_{29}- and O_{38}- and increasing at N_6-, O_{21}- and O_{31}- atoms. When, the proton transfer from O_{21}- atom to N_{18}- atom in the case of (**2**) to (**5**) is considered by decreasing net atomic charges at S_4-, S_{12}-, N_{18}-, O_{22}-, O_{29}-, and O_{31}- and increasing at N_6-, N_9-, O_{21}-, O_{34}- and O_{38}- atoms.

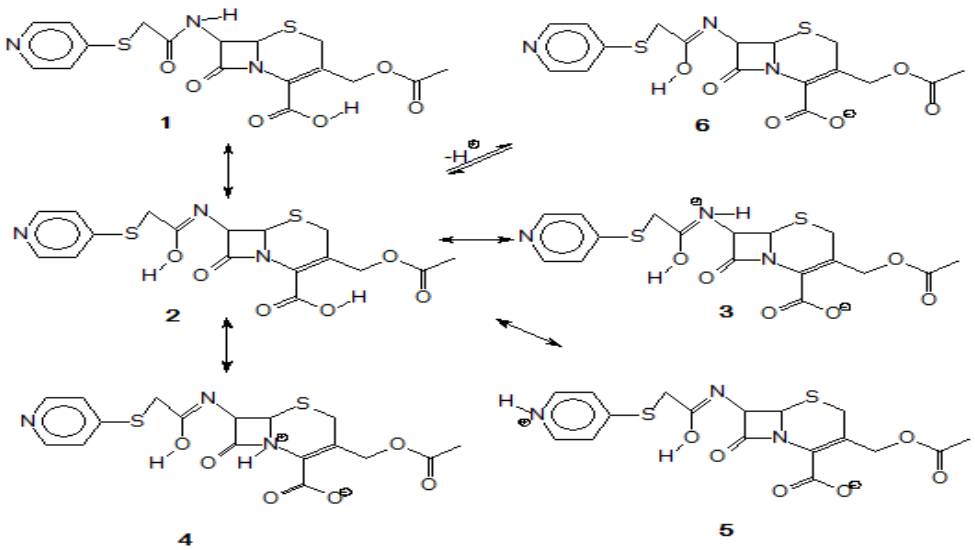
Equilibrium is normally established in polar solvents by rapid inter- or intra-molecular proton transfer from O_{21}-atom to N_9-, N_6- and N_{18}- atoms of cephapirin-lactim (**2**) as shown in Scheme-3. When one zwitterion is formed predominantly in a polar solution, its conformation can be assigned by comparison of its geometry and electronic structure. The position of proton transfer equilibrium can be affected by the nature of the solvent and concentration of the solution. The proton affinity (PA)[75] values for the different nitrogen atoms of neutral cephapirin-lactim RH (**2**) were calculated by using the equation (4). The proton affinity is in the order of N_9 (-63.586 kcal/mol) > N_6 (-109.4989 kcal/mol) > N_{18} (-43.6885 kcal/mol). However, zwitterion (**3**) appears to be more stable. Zwitterions are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium.

9.2. Computational study on conformations of cephapirin-lactim zwitterions

The spatial arrangement of atoms in a molecule is considered to study the conformations of cephapirin (**1**), and its enol form (**2**), zwitterions (**3**, **4** & **5**) and anion (**6**) with a view to

investigate molecular deformations. These can exist in *anti*- or *syn*- conformation, according to the position of atoms. In this context, the change in energy content of the zwitterion may depend on the changes in the parameters of dihedral angles. Figure-1 illustrates the atomic numbering of cephapirin-lactim (**2**). Fully optimized AM1 calculations scrutinize only the main data of dihedral angles (Table-VIII) of molecules (**1** to **6**) for the sake of simplicity. It has been found that the length of N_{18}-H bond (1.0416 A^0) in $RH^{\pm}$ ($N_{18}H^{\pm}$) (**5**) system is larger than that of N_9-H bond (1.0064 A^0) in $RH^{\pm}$ ($N_9H^{\pm}$) (**3**) and N_6-H bond (0.9848 A^0) in $RH^{\pm}$ ($N_6H^{\pm}$) (**4**) system.

From the Table-VIII and Scheme - 3, cephapirin-lactim (**2**) is formed by the migration of proton from N_9- atom to O_{31}-atom of cephapirin (**1**) through the lactam-lactim tautomerism, with the formation of O_{31}-H_{30} bond (0.9717 A^0) along with increasing bond length of O_{31}-C_{10} by 0.1373 A^0 and decreasing bond length of C_{10}-N_9 by 0.0875 A^0. The change of conformation from *–sc* of $C_{10}N_9C_7C_5$ and $C_{15}C_{13}S_{12}C_{11}$, *+ap* of $C_{19}C_1C_2C_3$ and $C_{24}C_{23}O_{22}C_{20}$ are changed to *–ap* conformation. *–sc* of $C_{13}S_{12}C_{11}C_{10}$ and *+ac* of $O_{22}C_{20}C_2C_3$ are changed to *+sc* conformation. Dihedral angle of $C_{14}C_{13}S_{12}C_{11}$, $C_{23}O_{22}C_{20}C_2$ and $O_{31}C_{10}N_9C_7$ are changed with conformation, *+ac* to *+sp*, *-sc* to *–ac* and *+sp* to *–sp* respectively to form stable lactim-form (2) and rest of positions have moderate changes.



Scheme - 3

The zwitterion $RH^{\pm}$ ($N_9H^{\pm}$) (**3**) is formed by the proton transfer between O_{21}-atom to N_9-atom of lactim-form RH (**2**) with the change of conformation from *-sc* of $S_{12}C_{11}C_{10}N_9$, *-ap* of $C_{10}N_9C_7C_5$ and $C_{15}C_{13}S_{12}C_{11}$ are changed to *-ac* conformation. *–ap* of $C_{11}C_{10}N_9C_7$ and $C_{20}C_2C_3C_1$ are changed to *+ap* conformation. Dihedral angle of $C_{14}C_{13}S_{12}C_{11}$ is changed to

+*sp* to +*sc* conformation to form more stable zwitterion and all other positions are not as much of altered. If the transfer of proton between O_{21}-atom to N_6-atom of lactim-form RH (**2**) forms the zwitterion $RH^{\pm}$ ($N_6H^{\pm}$) (**4**), with the change of dihedral angle of $H_{30}O_{31}C_{10}N_9$, $C_{11}C_{10}N_9C_7$ and $C_{20}C_2C_3C_1$ of respective +*sp*, -*ap*, -*ap* to +*ap* conformation. Dihedral angle of $S_{12}C_{11}C_{10}N_9$ and $C_{15}C_{13}S_{12}C_{11}$ of respective –*sc* and –*ap* to –*ac* conformation. The conformation of $C_{14}C_{13}S_{12}C_{11}$ and $O_{22}C_{20}C_2C_3$ are changed to respective +*sp* to +*sc* and +*sc* to +*ac* conformations to form stable zwitterion and all other positions are altered insignificant. If the transfer of proton between O_{21}- atom to N_{18}-atom of lactim-form RH (**2**) forms the zwitterion $RH^{\pm}$ ($N_{18}H^{\pm}$) (**5**), with the change of dihedral angle of -*ap* of $C_{11}C_{10}N_9C_7$ and $C_{19}C_1C_2C_3$, +*sc* of $O_{22}C_{20}C_2C_3$ are changed to +*ap* conformation. Dihedral angle of –*sc* of $S_{12}C_{11}C_{10}N_9$ and –*ap* of $C_{15}C_{13}S_{12}C_{11}$ are changed to -*ac* conformation. The change of conformations of $C_{14}C_{13}S_{12}C_{11}$ and $C_{23}O_{22}C_{20}C_2$ are converted respectively from +*sp* to +*sc* and –*ac* to -*sc* conformations and rest of positions have moderate changes. It can be concluded that the anion R^- (**6**) is formed with the removal of a proton on O_{21}- atom of cephapirin-lactim (**2**), and the change of conformation from -*ap* of $C_{11}C_{10}N_9C_7$, $C_{20}C_2C_3C_1$ and $C_{24}C_{23}O_{22}C_{20}$ are changed to +*ap* conformation. Dihedral angle of $S_{12}C_{11}C_{10}N_9$, $C_{14}C_{13}S_{12}C_{11}$ and $C_{15}C_{13}S_{12}C_{11}$ are changed the conformations from -*sc* to –*ac*, +*sp to* +*ac* and -*ap* to -*sc* respectively to form stable anion R^- (**6**) and rest of positions have moderate changes.

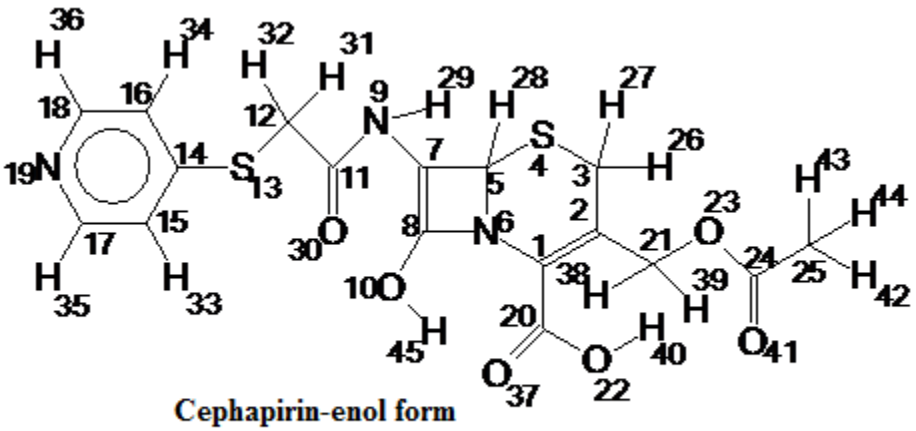
Table – VIII: Dihedral angle (°) of cephapirin (1), lactim-form of cephapirin (2), its zwitterions (3, 4 & 5) and anion (6) from AM1 calculation.

Dihedral angle (°)	RH (1)		RH (2)		$RH^{\pm}$ (3)		$RH^{\pm}$ (4)		$RH^{\pm}$ (5)		R^- (6)	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$H_{30}O_{31}C_{10}N_9$	-	-	+11.59	+*sp*	+18.03	+*sp*	+161.34	+*ap*	+14.97	+*sp*	+23.64	+*sp*
$O_{31}C_{10}N_9C_7$	+2.16	+*sp*	-1.59	-*sp*	-14.07	-*sp*	-3.46	-*sp*	-8.65	-*sp*	-7.55	-*sp*
$C_{10}N_9C_7C_5$	-35.44	-*sc*	-150.86	-*ap*	-147.98	-*ac*	-177.37	-*ap*	-151.22	-*ap*	-154.14	-*ap*
$C_{11}C_{10}N_9C_7$	-176.78	-*ap*	-179.23	-*ap*	+165.89	+*ap*	+178.69	+*ap*	+171.13	+*ap*	+175.19	+*ap*
$S_{12}C_{11}C_{10}N_9$	-32.88	-*sc*	-84.25	-*sc*	-90.94	-*ac*	-111.77	-*ac*	-91.29	-*ac*	-101.79	-*ac*
$C_{13}S_{12}C_{11}C_{10}$	-79.79	-*sc*	+78.83	+*sc*	+74.12	+*sc*	+63.32	+*sc*	+41.79	+*sc*	+53.49	+*sc*
$C_{14}C_{13}S_{12}C_{11}$	+139.89	+*ac*	+24.79	+*sp*	+67.34	+*sc*	+83.01	+*sc*	+53.31	+*sc*	+116.56	+*ac*
$C_{15}C_{13}S_{12}C_{11}$	-45.08	-*sc*	-160.13	-*ap*	-118.13	-*ac*	-97.45	-*ac*	-125.99	-*ac*	-69.29	-*sc*
$C_{19}C_1C_2C_3$	+178.43	+*ap*	-179.21	-*ap*	-173.26	-*ap*	-160.41	-*ap*	+173.65	+*ap*	-176.26	-*ap*
$C_{20}C_2C_3C_1$	-178.07	-*ap*	-178.22	-*ap*	+178.56	+*ap*	+171.19	+*ap*	-179.85	-*ap*	+179.7	+*ap*
$O_{22}C_{20}C_2C_3$	+104.19	+*ac*	+74.85	+*sc*	+60.02	+*sc*	+148.14	+*ac*	+153.20	+*ap*	+67.86	+*sc*
$C_{23}O_{22}C_{20}C_2$	-82.31	-*sc*	-91.85	-*ac*	-92.87	-*ac*	-94.52	-*ac*	-56.54	-*sc*	-90.38	-*ac*
$C_{24}C_{23}O_{22}C_{20}$	+179.81	+*ap*	-175.17	-*ap*	-174.33	-*ap*	-178.21	-*ap*	-177.63	-*ap*	+177.67	+*ap*
$H_{37}O_{21}C_{19}C_1$	+178.53	+*ap*	+178.15	+*ap*	-	-	-	-	-	-	-	-
$HN_9C_7C_5$	-	-	-	-	+17.69	+*sp*	-	-	-	-	-	-
$HN_6C_5S_4$	-	-	-	-	-	-	+73.07	+*sc*	-	-	-	-
$HN_{18}C_{16}C_{14}$	-	-	-	-	-	-	-	-	+166.78	+ap	-	-

* Conformational analyses using prefixes a = anti, s = syn, p = periplanar, c=clinal, and + & -signs.[17]

10. Computational study on cephapirin keto-enol zwitterions[82]

The present investigation focuses on the evaluation of the significance of the molecular conformation and electronic properties of cephapirin (**1**) and its enol-form (**2**), zwitterions $RH^{\pm}$ (**3, 4 & 5**) and Anion (**6**). The mechanism of proton transfer in cephapirin enol-form has been studied by the different positions of net charges on nitrogen atoms in the molecule. Taking cephapirin enol-form (**2**), as a neutral molecule and the conformation and electronic structures of zwitterions $RH^{\pm}$ (**3,4 & 5**) system, in which are included $RH^{\pm}$ ($N_{19}H^{\pm}$) (**3**), $RH^{\pm}$ ($N_6H^{\pm}$) (**4**) and $RH^{\pm}$ ($N_9H^{\pm}$) (**5**) have been determined by full optimization calculations, using semi-empirical molecular orbital AM1 method.



Cephapirin-enol form

Figure - 3

10.1. Computational study on electronic structure of cephapirin keto-enol zwitterions

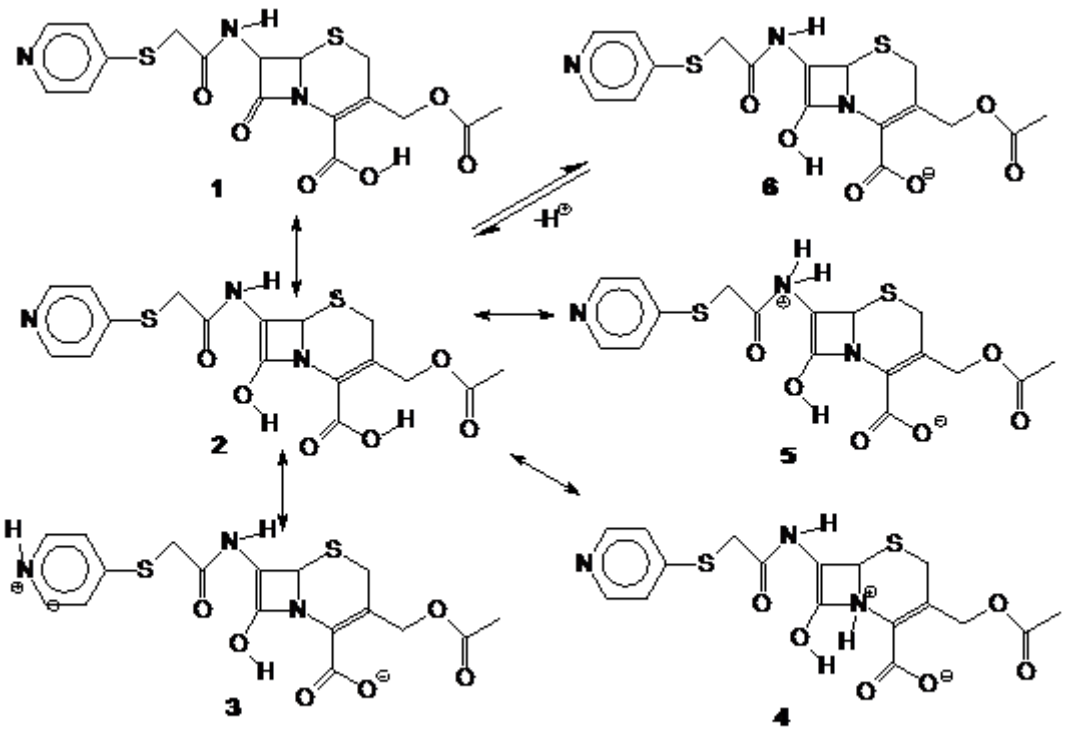
Cephapirin may undergo keto-enol tautomerism and exists as cephapirin (1) and cephapirin enol-form (2) along with its zwitterions $RH^{\pm}$ (3, 4 and 5) and anion (6) as shown in Scheme-4. In this context, the numbering of the system is shown in Figure–3. The calculated heats of formation (ΔH_f^o), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero-atoms of the molecules (1 to 6) are incorporated in Table-IX. The net charges on N_6-, N_9- and N_{19}- atoms are -0.1453, -0.2910 and -0.1156 respectively in the case of cephapirin enol-form (2). Usually, the nitrogen atom with larger negative value of net charge accepts proton more easily. Thus, the sequence of protonation for nitrogen atoms in cephapirin enol-form (2) is increasing in the order of $N_9 < N_6 < N_{19}$. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that promotion of an electron from HOMO to LUMO, in a photochemical reaction, antara-facial path way is allowed in case of anion (6) due to the presence of opposite sign and the supra-facial path way is

allowed in molecules (1, 2, 3, 4 and 5) due to the presence of same sign.[77] The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of molecules **2 < 1 < 4 < 3 < 5 < 6**. It is observed that anion of cephapirin enol-form (6) is higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[74] (μ_{ind}) of zwitterions can be estimated with respect to cephapirin enol-form (2) by using the equation (1) and observed in the order of $\Delta\mu_{ind}$ (4) 7.9545D < $\Delta\mu$ind (3) 10.5303D < $\Delta\mu$ind (5) 11.9369D. Thus obtained results reveal about electronic properties and reactivity of the molecule, which is depending upon conformational structure.

From the reactivity point of view, the searching of protonation sites is important in cephapirin enol-form, due to the presence of different positions of oxygen and nitrogen atoms. According to the heat of formation (ΔH_f^o) data, the stability of the compounds have decreased in the order of **6 < 1 < 2 < 4 < 3 < 5**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual zwitterions, ions or molecules. Zwitterions are formed with the difference in the heat of formation (ΔH_f^o) of +47.4440 kcal/mol, +42.1344 kcal/ mol and +64.3675 kcal/ mol respectively in the conversion of (2) to (3), (2) to (4) and (2) to (5).

Table –IX:	**Heat of formation (ΔH_f^o in kcal/mol), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV) and the atomic charges on S_4, S_{13}, N_6, N_9, N_{19}, O_{10}, O_{22}, O_{23}, O_{30}, O_{37} and O_{41} of Cephapirin (1) and its enol-form (2) along with zwitterions (3, 4 & 5) and anion (6) from AM1 calculation.**					
Parameters	**RH (1)**	**RH (2)**	**$RH^{\pm}$ (3)**	**$RH^{\pm}$ (4)**	**$RH^{\pm}$ (5)**	**R^- (6)**
ΔH_f^0 (kcal/mol)	-139.3941	-113.6674	-66.2234	-71.5330	-49.2999	-152.7934
μ (Debye)	1.6913	0.8333	11.3656	8.7878	12.7702	13.4259
E_{HOMO} (eV)	-9.098	-8.865	-8.269	-9.400	-8.979	-5.621
E_{LUMO} (eV)	-6.700	-0.763	-2.226	-1.289	-2.026	+1.279
S_4 (atomic charge)	+0.0706	+0.0945	+0.0998	+0.2084	+0.0589	+0.0736
S_{13} (atomic charge)	+0.2261	+0.2350	+0.3328	+0.2733	+0.2477	+0.1972
N_6 (atomic charge)	-0.2357	-0.1453	-0.1860	+0.0304	-0.1534	-0.1542
N_9 (atomic charge)	-0.3626	-0.2910	-0.2287	-0.2886	+0.0675	-0.2660
N_{19} (atomic charge)	-0.1255	-0.1156	-0.0835	-0.1249	-0.1061	-0.1266
O_{10} (atomic charge)	-0.2521	-0.2052	-0.2280	-0.1931	-0.2360	-0.8570
O_{22} (atomic charge)	-0.2796	-0.3144	-0.5778	-0.5853	-0.5978	-0.5744
O_{23} (atomic charge)	-0.2766	-0.2721	-0.2553	-0.2833	-0.2538	-0.2812
O_{30} (atomic charge)	-0.3341	-0.3242	-0.2750	-0.3192	-0.2182	-0.3220
O_{37} (atomic charge)	-0.3478	-0.3448	-0.5589	-0.4276	-0.4438	-0.5132
O_{41} (atomic charge)	-0.3601	-0.3601	-0.3797	-0.3538	-0.3837	-0.3749

It can be predicted that the conversion of cephapirin enol-form (2) to zwitterion (4) is lower energy process than the conversion of (2) to (3) and (2) to (5). The protonation site of cephapirin (2), containing N_9- atom is predicted to be the main basic centre of molecule (2). However, negative atomic charges are also present on the other atoms of the molecule. The proton shifting from O_{22}- atom to N_{19}- atom in the case of cephapirin enol-form (2) to zwitterion (3) is considered by decreasing net atomic charges at S_4-, S_{13}-, N_9-, N_{19}-, O_{23}- and O_{30}- and increasing at N_6-, O_{10}-, O_{22}-, O_{37}- and O_{41}- atoms. The proton shifting from O_{22}- atom to N_6- atom in the case of (2) to (4) is considered by decreasing net atomic charges at S_4-, S_{13}-, N_6-, N_9-, O_{10}-, O_{30}- and O_{41}- and increasing at N_{19}-, O_{22}-, O_{23}- and O_{37}- atoms. When, the proton transfer from O_{22}- atom to N_9- atom in the case of (2) to (5) is considered by decreasing net atomic charges at S_{13}-, N_9-, N_{19}-, O_{23}- and O_{30}- and increasing at S_4-, N_6-, O_{10}-, O_{22}-, O_{37}- and O_{41}- atoms. Equilibrium is normally established in polar solvents by rapid inter- or intra-molecular proton transfer from O_{22}-atom to N_6-, N_9- and N_{19}- atoms of cephapirin enol-form as shown in Scheme - 4. The conformation of one zwitterion is predominantly formed in a polar solution and it can be assigned by comparison of its geometry and electronic structure. The position of proton transfer equilibrium can be affected by the nature of the solvent and concentration of the solution. The proton affinity (PA)[72] values for the different nitrogen atoms of cephapirin enol- form RH (2) were calculated by using the equation (4). The proton affinity is to be predicted in the order of N_9-atom (-64.3675 kcal/mol) < N_{19}–atom (-47.4440 kcal/mol) < N_6–atom (-42.1344 kcal/mol). However, zwitterion (**4**) appears to be more stable. Cephapirin[6] is unstable in aqueous solutions and it is often directed to keep injectable preparations. The pKa of cephapirin is HA = 2.67 and HB+ = 4.49. Zwitterions are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium.



Scheme - 4

10.2. Computational study on conformations of cephapirin-enol zwitterions

The spatial arrangement of atoms in a molecule is considered to study the conformations of of cephapirin enol-form RH (2), zwitterions $RH^{\pm}$(3, 4 & 5) and anion R^{-} (6) with a view to undergo molecular deformations. Zwitterions can exist in anti- or syn- conformation, according to the position of atoms. In this context, the change in energy content of the zwitterion may depend on the changes in the parameters of dihedral angles. The conformational analyses of zwitterions reveal about molecular deformations. Figure-3 illustrates the atomic numbering of cephapirin enol-form (2). For the sake of simplicity, fully optimized AM1 calculations were scrutinized only the main data of dihedral angles of (1 to 6), which are included in Table - X. This study has concluded the proton transfer sequence in nitrogen atoms of cephapirin enol-form (2) and also agreed, well with the attained results. Thus, the stable the conformations of zwitterions (**3, 4** and **5**) were formed with the proton transfer to alternate nitrogen atom of the molecule.

From the Table-X and Scheme-4, it can be found that the anion R^{-} (**6**) is formed with the removal of a proton on O_{22}- atom of cephapirin enol-form RH (**2**), the conformations of ***-ac*** of $C_{11}N_9C_7C_5$ and ***+sc*** of $C_{15}C_{14}S_{13}C_{12}$ are changed to ***+ac***. Dihedral angle of $C_{21}C_2C_3C_1$, $C_{23}C_{21}C_2C_3$, and $C_{24}O_{23}C_{21}C_2$ are changed with conformations**,** ***-ap*** to ***+ap,*** ***+ac*** to ***+sc*** and

+sc to ***–ac*** respectively to form stable anion and rest of positions have moderate changes. The zwitterion $RH^{\pm}$ ($N_{19}H^{\pm}$) (**3**) is formed by the proton transfer between O_{22}- atom to N_{19}-atom of RH (**2**) with respective conformations ***-sp*** of $O_{30}C_{11}N_9C_7$, ***+ap*** of $C_{12}C_{11}N_9C_7$ and ***+sc*** of $C_{14}S_{13}C_{12}C_{11}$, which are changed to ***-sc, +ac*** and***d –sc*** conformations. Dihedral angle of ***+sc*** of $C_{15}C_{14}S_{13}C_{12}$, ***+ac*** of $C_{16}C_{14}S_{13}C_{12}$ and ***+ap*** of $C_{20}C_1C_2C_3$ are changed to respective ***+ap, -sp*** and ***-ap*** conformations. The conformations are changed from ***-ap*** to ***+ap***, and ***+sc*** to ***-sp*** in case of $C_{25}C_{24}O_{23}C_{21}$ and $H_{45}O_{10}C_8N_6$ respectively to form more stable zwitterion and all other positions are not as much of altered. The protonation on N_{19}- atom is observed ***+ap*** conformation with the formation of $HN_{19}C_{17}C_{15}$ dihedral angle.

Table-X:	**Dihedral angle (o) of Cephapirin (1) and its enol-form (2) along with zwitterions (3, 4, 5) and anion (6) from AM1 calculation**											
Dihedral anzgle (o)	**RH(1)**		**RH(2)**		**$RH^{\pm}$(3)**		**$RH^{\pm}$(4)**		**$RH^{\pm}$(5)**		**R^-(6)**	
	Angle	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**
$O_{30}C_{11}N_9C_7$	+2.16	+*sp*	-2.03	-*sp*	-50.53	-*sc*	-173.69	-*ap*	+179.82	+*ap*	-10.83	-*sp*
$C_{11}N_9C_7C_5$	-35.44	-*sc*	-128.30	-*ac*	-147.06	-*ac*	-85.63	-*sc*	-83.31	-*sc*	+104.37	+*ac*
$C_{12}C_{11}N_9C_7$	-176.78	-*ap*	+179.81	+*ap*	+127.68	+*ac*	+9.49	+*sp*	+1.28	+*sp*	+170.91	+*ap*
$S_{13}C_{12}C_{11}N_9$	-32.88	-*sc*	-69.91	-*sc*	-57.61	-*sc*	-76.54	-*sc*	-102.55	-*ac*	-82.52	-*sc*
$C_{14}S_{13}C_{12}C_{11}$	-79.79	-*sc*	+86.54	+*sc*	--39.21	-*sc*	+124.46	+*ac*	+142.19	+*ac*	+74.85	+*sc*
$C_{15}C_{14}S_{13}C_{12}$	+139.89	+*ac*	+69.19	+*sc*	+160.13	+*ap*	+52.22	+*sc*	+96.05	+*ac*	+102.71	+*ac*
$C_{16}C_{14}S_{13}C_{12}$	-45.08	-*sc*	-117.14	-*ac*	-16.08	-*sp*	-131.25	-*ac*	-88.68	-*sc*	-83.33	-*sc*
$C_{20}C_1C_2C_3$	+178.43	+*ap*	+172.04	+*ap*	-171.83	-*ap*	-175.93	-*ap*	+178.38	+*ap*	+174.16	+*ap*
$C_{21}C_2C_3C_1$	-178.07	-*ap*	-179.20	-*ap*	-179.59	-*ap*	-179.83	-*ap*	-179.48	-*ap*	+178.50	+*ap*
$O_{23}C_{21}C_2C_3$	+104.19	+*ac*	+110.12	+*ac*	+142.08	+*ac*	+34.24	+*sc*	+82.41	+*sc*	+47.64	+*sc*
$C_{24}O_{23}C_{21}C_2$	-82.31	-*sc*	-80.60	-*sc*	-78.92	-*sc*	-102.47	-*ac*	-85.55	-*sc*	-102.29	-*ac*
$O_{25}C_{24}O_{23}C_{21}$	+179.81	+*ap*	-179.45	-*ap*	+173.99	+*ap*	-170.16	-*ap*	-176.94	-*ap*	-179.24	-*ap*
$H_{40}O_{22}C_{20}C_1$	+178.53	+*ap*	-179.82	-*ap*	-		-	-	-	-	-	-
$H_{45}O_{10}C_8N_6$	-	-	+44.12	+*sc*	-29.14	-*sp*	+4.39	+*sp*	-2.82	-*sp*	-9.91	-*sp*
$HN_6C_5S_4$	-	-	-	-	-		+138.14	+*ac*	-	-	-	-
$HN_9C_7C_5$	-	-	-	-	-		-	-	+154.81	+*ap*	-	-
$HN_{19}C_{17}C_{15}$	-	-	-	-	+166.69	+*ap*	-	-	-	-	-	-

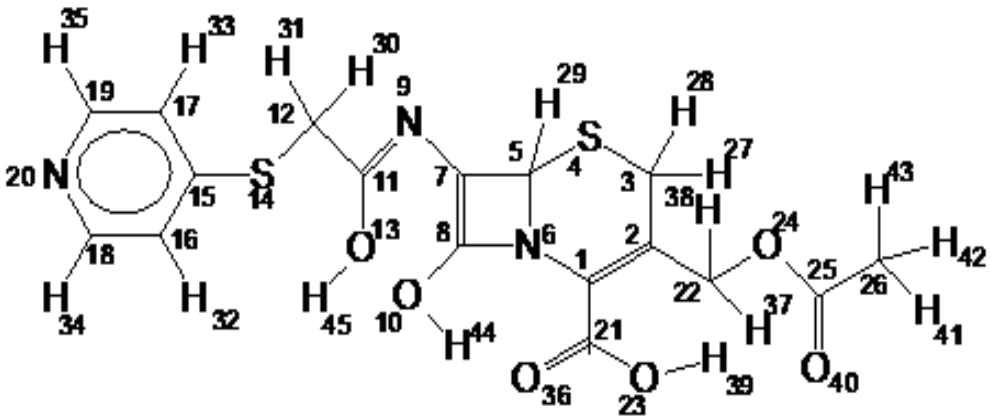
* Conformational analyses using prefixes a = anti, s = syn, p = peri-planar, c = clinal, and + & - signs[17].

If the zwitterion $RH^{\pm}$ ($N_6H^{\pm}$) (**4**) is formed by transfer of proton between O_{22}-atom to N_6-atom of RH (**2**), the dihedral angle of ***-sp*** of $O_{30}C_{11}N_9C_7$, ***-ac*** of $C_{11}N_9C_7C_5$, and ***+ap*** of $C_{12}C_{11}N_9C_7$ are changed to respective ***–ap, -sc*** and ***+ap*** conformations. Dihedral angle of $C_{20}C_1C_2C_3$, $O_{23}C_{21}C_2C_3$ and $H_{45}O_{10}C_8N_6$ are changed conformations, ***+ap*** to ***–ap,*** +ac to ***+sc*** and ***+sc*** to ***+sp*** respectively to form stable zwitterion and all other positions are altered insignificant. N_6-atom is protonated with ***+ac*** conformation in the case of $HN_6C_5S_4$ dihedral angle. If the transfer of proton between O_{22}-atom to N_9-atom of RH (**2**), thus formed the

zwitterion $RH^{\pm}$ ($N_9H^{\pm}$) (**5**), is investigated the dihedral angle of ***-sp*** of $O_{30}C_{11}N_9C_7$, ***-ac*** of $C_{11}N_9C_7C_5$, ***+ap*** of $C_{12}C_{11}N_9C_7$ and ***-sc*** of $S_{13}C_{12}C_{11}N_9$, which are changed to ***+ap, -sc, +sp*** and ***–ac*** conformations respectively. Dihedral angle of $C_{14}S_{13}C_{12}C_{11}$, $C_{15}C_{14}S_{13}C_{12}$, $C_{16}C_{14}S_{13}C_{12}$ and $H_{45}O_{10}C_8N_6$ are changed the conformations, ***+sc to +ac, +sc to +ac, -ac to -sc*** and ***+sc*** to ***-s***p respectively to form stable zwitterion and rest of positions have moderate changes. It is also observed that N_9-atom is protonated with ***+ap*** conformation in the case of $HN_9C_7C_5$ dihedral angle.

11. Computational study on cephapirin lactim-enol zwitterions[83]

In this study, it is calculated and interpreted for cephapirin (**1**) and its lactim-enol-form (**2**), zwitterions $RH^{\pm}$ (**3**, **4** & **5**) and Anion (**6**). The mechanism of proton transfer in cephapirin lactim-enol has been studied by the different positions of net charges on nitrogen atoms in the molecule. Taking cephapirin lactim-enol (**2**), as a neutral molecule and the conformation and electronic structures of zwitterions $RH^{\pm}$ (**3**,**4** & **5**) system, in which are included $RH^{\pm}$ ($N_{20}H^{\pm}$) (**3**), $RH^{\pm}$ ($N_9H^{\pm}$) (**4**) and $RH^{\pm}$ ($N_6H^{\pm}$) (**5**) have been determined by full optimization calculations, using semi-empirical molecular orbital AM1 method. The aim of this study is to not only clarify the characterization of molecules but show the way to future studies of this molecule.



Cephapirin lactim-enol form

Figure - 4

11.1. Computational study on electronic structure of cephapirin lactim-enol zwitterions

Cephapirin may undergo keto-enol and lactam-lactim tautomerism simultaneously to form lactim-enol of cephapirin (**2**). Thus formed cephapirin lactim-enol form (**2**) may undergo

zwitterions $RH^{\pm}$ (**3, 4** and **5**) and anion (**6**) in the polar medium as shown in Scheme-5. In this context, the numbering of cephapirin lactim-enol form (**2**) is shown in Figure – 4. The calculated heats of formation (ΔH_f^o), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero-atoms of the molecules (**1** to **6**) are incorporated in Table-XI. The net charges on N_6-, N_9- and N_{20}- atoms are -0.1108, -0.1453 and -0.1374 respectively in the case of cephapirin lactim-enol form (**2**). Usually, the nitrogen atom with larger negative value of net charge accepts proton more easily. Thus, the sequence of protonation for nitrogen atoms in cephapirin lactim-enol form (**2**) is increasing in the order of $N_6 < N_9 < N_{20}$. The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that promotion of an electron from HOMO to LUMO, in a photochemical reaction, antara-facial path way is allowed in case of anion (**6**) due to the presence of opposite sign and the supra-facial path way is allowed in molecules (**1, 2, 3, 4** and **5**) due to the presence of same sign.[77] As per electron excitation energies (ΔE, in eV), it is predicted that the reactivity of zwitterions is decreased in the order of **5** > **4** > **3**. It is also evidenced that zwitterion $RH^{\pm}$ ($N_{20}H^{\pm}$) (**3**) is more stable than other zwitterions. The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of molecules **1** < **5** < **2** < **4** < **3** < **6**. It is observed that anion of cephapirin lactim-enol form (**6**) is higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[74] (μ_{ind}) of all systems can be estimated with respect to cephapirin (**1**) by using the equation (1) and found in the order of $\Delta\mu_{ind}$(**5**) 0.7478D < $\Delta\mu_{ind}$(**2**) 2.1592D < $\Delta\mu_{ind}$(**4**) 6.8866D < $\Delta\mu_{ind}$(**3**) 10.4838D < $\Delta\mu_{ind}$(**6**) 15.588D. Thus obtained results reveal about electronic properties and reactivity of the molecule, which is depending upon conformational structure. From the reactivity point of view, the searching of protonation sites is important in cephapirin lactim-enol, due to the presence of different positions of oxygen and nitrogen atoms.

Table –XI	**Heat of formation (ΔH_f^o in kcal/mol), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV) and the atomic charges on S_4, S_{14}, N_6, N_9, N_{20}, O_{10}, O_{13}, O_{23}, O_{24}, O_{36} and O_{40} of Cephapirin (1) and its lactim-enol form (2) along with zwitterions (3, 4 & 5) and anion (6) from AM1 calculation.**					
Parameters	**RH (1)**	**RH (2)**	**$RH^{\pm}$ (3)**	**$RH^{\pm}$ (4)**	**$RH^{\pm}$ (5)**	**R^- (6)**
ΔH_f^0 (kcal/mol)	-139.3941	-107.3375	-67.5049	-99.6132	-67.8850	-132.9549
μ (Debye)	1.6913	3.8505	12.1751	8.5779	2.4391	17.2793
E_{HOMO} (eV)	-9.098	-8.562	-8.278	-8.471	-9.393	-5.264
E_{LUMO} (eV)	-6.700	-0.693	-2.048	-1.425	-1.329	+1.221
Electronic Excitation Energy (ΔE, in eV)	2.398	7.869	6.230	7.046	8.064	6.485
S_4 (atomic charge)	+0.0706	+0.1304	-0.0007	+0.0411	+0.1866	+0.0391
S_{14} (atomic charge)	+0.2261	+0.2376	+0.2737	+0.1828	+0.2462	+0.1407
N_6 (atomic charge)	-0.2357	-0.1108	-0.0898	-0.2437	+0.0357	-0.1261
N_9 (atomic charge)	-0.3626	-0.1453	-0.1779	+0.1099	-0.1315	-0.0852
N_{20} (atomic charge)	-0.1255	-0.1374	-0.0504	-0.1075	-0.1337	-0.1278
O_{10} (atomic charge)	-0.2521	-0.2007	-0.1498	-0.3269	-0.1879	-0.1692
O_{13} (atomic charge)	-0.3341	-0.2825	-0.2764	-0.2341	-0.2925	-0.2903
O_{23} (atomic charge)	-0.2796	-0.2831	-0.6333	-0.3169	-0.5740	-0.5448
O_{24} (atomic charge)	-0.2766	-0.2757	-0.2733	-0.2893	-0.2902	-0.2837
O_{36} (atomic charge)	-0.3478	-0.3480	-0.4538	-0.2801	-0.4394	-0.5151
O_{40} (atomic charge)	-0.3601	-0.3563	-0.4171	-0.3353	-0.3440	-0.3698

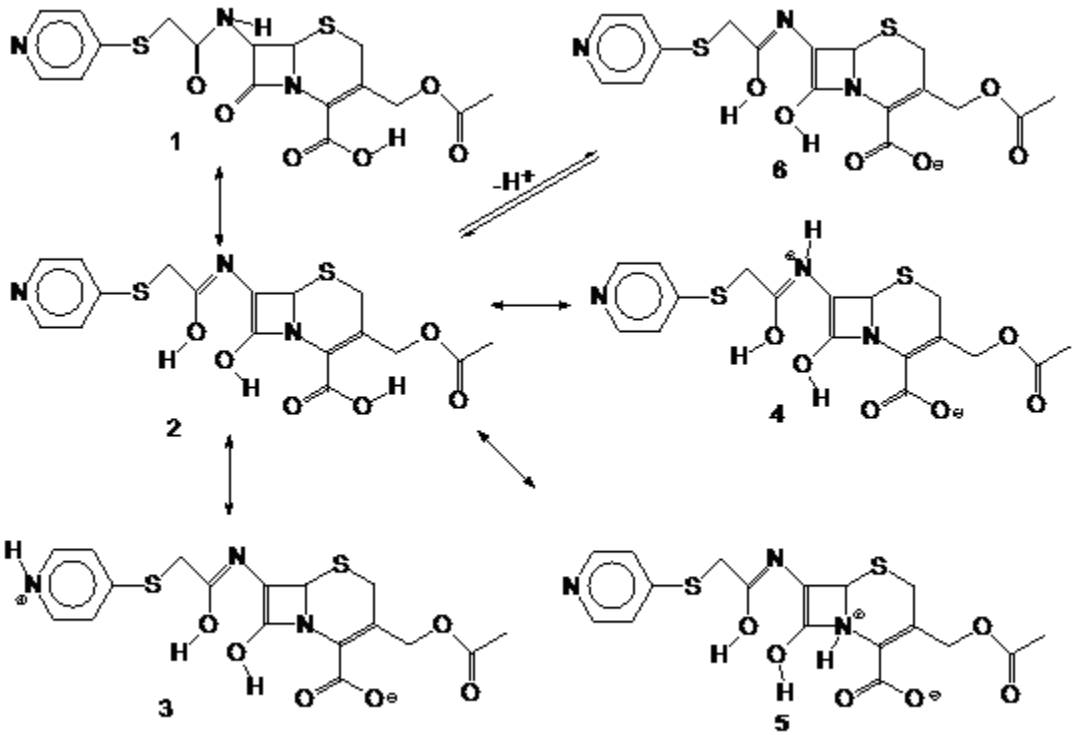
According to the heat of formation (ΔH_f^o) data, the stability of the compounds have increased in the order of **3** < **5** < **4** < **2** < **6** < **1**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual zwitterions, ions or molecules. Zwitterions are formed with the difference in the heat of formation (ΔH_f^o) of +39.8326 kcal/mol, +7.7243 kcal/ mol and +39.4525 kcal/ mol respectively in the conversion of (**2**) to (**3**), (**2**) to (**4**) and (**2**) to (**5**). It can be predicted that the conversion of cephapirin lactim-enol form (**2**) to zwitterion (**4**) is lower energy process than the conversion of (**2**) to (**3**) and (**2**) to (**5**). The protonation site of cephapirin lactim-enol form (**2**), containing N_9- atom is predicted to be the main basic centre of molecule (**2**). However, negative atomic charges are also present on the other atoms of the molecule. The proton shifting from O_{23}- atom to N_{20}- atom in the case of cephapirin enol-form (**2**) to zwitterion (**3**) is considered by decreasing net atomic charges at S_{14}-, N_6-, N_{20}-, O_{10}-, O_{13}- and O_{24}- and increasing at S_4-, N_9-, O_{23}-, O_{36}- and O_{40}- atoms. The proton shifting from O_{23}- atom to N_9- atom in the case of (**2**) to (**4**) is considered by decreasing net atomic charges at N_9-, N_{20}-, O_{13}-, O_{36}- and O_{40}- and increasing at S_4-, S_{14}-, N_6-, O_{10}-, O_{23}- and O_{24}- atoms. When, the proton transfer from O_{23}- atom to N_6- atom in the case of (**2**) to (**5**) is considered by decreasing net atomic charges at S_4-,S_{14}-, N_6-, N_9-, N_{20}-, O_{10}- and O_{40}- and increasing at O_{13}-,

O_{23}-, O_{24}- and O_{36}- atoms. Equilibrium is normally established in polar solvents by rapid inter- or intra-molecular proton transfer from O_{23}-atom to N_6-, N_9- and N_{20}- atoms of cephapirin lactim-enol form as shown in Scheme-5. The conformation of one zwitterion is predominantly formed in a polar solution and it can be assigned by comparison of its geometry and electronic structure. The position of proton transfer equilibrium can be affected by the nature of the solvent and concentration of the solution. The proton affinity (PA)[75] values for the different nitrogen atoms of cephapirin lactim-enol form RH (**2**) were calculated by using the equation (4). The proton affinity is to be predicted in the order of N_{20}-atom (-39.8326 kcal/mol) < N_6–atom (-39.4525 kcal/mol) < N_9–atom (-7.7243 kcal/mol). However, zwitterion (**4**) appears to be more stable. Cephapirin[6] is unstable in aqueous solutions and it is often directed to keep injectable preparations. The pKa of cephapirin is HA = 2.67 and HB^+ = 4.49. Zwitterions are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium.

11.2. Computational study on conformations of cephapirin lactim-enol zwitterions

The spatial arrangement of atoms in a molecule is considered to study the conformations of cephapirin lactim-enol form (**2**), zwitterions (**3**, **4** & **5**) and anion (**6**) with a view to undergo molecular deformations. Zwitterions can exist in *anti*- or *syn*- conformation, according to the position of atoms. In this context, the change in energy content of the zwitterion may depend on the changes in the parameters of dihedral angles. The conformational analyses of zwitterions reveal about molecular deformations. Figure-4 illustrates the atomic numbering of cephapirin lactim-enol form (**2**). For the sake of simplicity, fully optimized AM1 calculations were scrutinized only the main data of dihedral angles of molecules (**1** to **6**), which are included in Table-XII. This study concluded that the proton transfer sequence in nitrogen atoms of cephapirin lactim-enol form (**2**) and also agreed well with the attained results. Thus, the stable conformations of zwitterions (**3**, **4** and **5**) were formed with the proton transfer to alternate nitrogen atom of the molecule. From the Table-XII and Scheme - 5, it can be found that the anion R^- (**6**) is formed with the removal of a proton on O_{23}- atom of cephapirin lactim-enol form RH (**2**), the conformations of +*ap* of $C_{12}C_{11}N_9C_7$ and $H_{45}O_{13}C_{11}N_9$ are changed to respective –*ap* and +*ac* conformations. Dihedral angle of $C_{16}C_{15}S_{14}C_{12}$, $C_{17}C_{15}S_{14}C_{12}$, and $O_{23}C_{21}C_1C_2$ are changed with conformations, +*sp* to +sc, -*ap* to -*ac* and +*ac* to -*sc* respectively to form stable anion and rest of positions have moderate changes. The zwitterion $RH^{\pm}$ ($N_{20}H^{\pm}$) (**3**) is formed by the proton transfer between O_{23}- atom to N_{20}-atom of RH (**2**) with respective conformations +*ap* of $O_{10}C_8N_6C_5$ and $C_{22}C_2C_3C_1$ are changed to –

ap conformation. -*ap* of $O_{17}C_{15}S_{14}C_{12}$ and +*ac* of $O_{23}C_{21}C_1C_2$ are changed to –*ac* conformation. +*sc* of $O_{24}C_{22}C_2C_3$ and -*ac* of $C_{25}O_{24}C_{22}C_2$ are changed to –*sc* conformation. Dihedral angle of +*sp* of $C_{16}C_{15}S_{14}C_{12}$ and +*ap* of $H_{45}O_{13}C_{11}N_9$ are changed to respective +*sc* and +*sp* conformations to form more stable zwitterion and all other positions are altered slightly. The protonation on N_{20}- atom is observed +*sp* conformation with the formation of $HN_{20}C_{19}C_{16}$ dihedral angle.



Scheme - 5

If the zwitterion $RH^{\pm}$ ($N_9H^{\pm}$) (**4**) is formed by transfer of proton between O_{23}-atom to N_9-atom of RH (**2**), the dihedral angle of +*sp* of $C_{16}C_{15}S_{14}C_{12}$, and +*ac* of $O_{23}C_{21}C_1C_2$ are changed to +*sc* conformation. +*ap* of $H_{44}O_{10}C_8N_6$, and +*sc* of $O_{24}C_{22}C_2C_3$ are changed to -*sp* conformation. Dihedral angles of $C_{17}C_{15}S_{14}C_{12}$, $H_{45}O_{13}C_{11}N_9$ and $C_{22}C_2C_3C_1$ are changed conformations, -*ap* to –*ac*, +*ap* to +*ac* and +*ap* to -*ap* respectively to form stable zwitterion and all other positions are altered insignificant. N_9-atom is protonated with -*ap* conformation in the case of $HN_9C_7C_5$ dihedral angle. If the transfer of proton between O_{23}-atom to N_6-atom of RH (**2**), thus formed the zwitterion $RH^{\pm}$ ($N_6H^{\pm}$) (**5**), is investigated the dihedral angle of +*ap* of $C_{12}C_{11}N_9C_7$, $C_{21}C_1C_2C_3$ and $C_{22}C_2C_3C_1$ are changed to –*ap* conformation. -*ap* of $O_{17}C_{15}S_{14}C_{12}$ and +*ac* of $O_{23}C_{21}C_1C_2$ are changed to +*ap* conformation. +*sc* of $O_{24}C_{22}C_2C_3$ and +*ap* of $H_{44}O_{10}C_8N_6$ are changed to +*sp* conformation. Dihedral angle of $C_{16}C_{15}S_{14}C_{12}$ is

changed the conformation +*sp* to -*sp* to form stable zwitterion and rest of positions with moderate changes. It is also observed that N_6-atom is protonated with +*ac* conformation in the case of $HN_6C_5S_4$ dihedral angle.

Table-XII	**Dihedral angle (°) of Cephapirin (1) and its lactim-enol form (2) along with zwitterions (3, 4, 5) and anion (6) from AM1 calculation.**											
Dihedral angle (°)	**RH (1)**		**RH (2)**		**$RH^{\pm}$ (3)**		**$RH^{\pm}$ (4)**		**$RH^{\pm}$ (5)**		**R^{-} (6)**	
	Angle	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**	**Angle**	**(*)**
$O_{10}C_8N_6C_5$	-178.79	***-ap***	+177.72	***+ap***	-169.67	***-ap***	+173.13	***+ap***	+177.12	***+ap***	+176.03	***+ap***
$O_{13}C_{11}N_9C_7$	+2.16	***+sp***	-2.97	***-sp***	-16.03	***-sp***	-5.81	***-sp***	-1.31	***-sp***	-5.63	***-sp***
$C_{11}N_9C_7C_5$	-35.44	***-sc***	-15.27	***-sp***	-4.48	***-sp***	-2.19	***-sp***	-26.27	***-sp***	-2.71	***-sp***
$C_{12}C_{11}N_9C_7$	-176.78	***-ap***	+179.65	***+ap***	+162.81	***+ap***	+172.66	***+ap***	-178.98	***-ap***	-179.63	***-ap***
$S_{14}C_{12}C_{11}N_9$	-32.88	***-sc***	-125.77	***-ac***	-91.04	***-ac***	-112.94	***-ac***	-132.32	***-ac***	-130.69	***-ac***
$C_{15}S_{14}C_{12}C_{11}$	-79.79	***-sc***	+76.22	***+sc***	+43.65	***+sc***	+82.24	***+sc***	+81.05	***+sc***	+80.67	***+sc***
$C_{16}C_{15}S_{14}C_{12}$	+139.89	***+ac***	+11.24	***+sp***	+41.41	***+sc***	+82.32	***+sc***	-3.63	***-sp***	+66.83	***+sc***
$C_{17}C_{15}S_{14}C_{12}$	-45.08	***-sc***	-171.74	***-ap***	-135.83	***-ac***	-101.79	***-ac***	+176.29	***+ap***	-116.18	***-ac***
$C_{21}C_1C_2C_3$	+178.43	***+ap***	+179.51	***+ap***	+167.94	***+ap***	+179.63	***+ap***	-176.71	***-ap***	+176.27	***+ap***
$C_{22}C_2C_3C_1$	-178.07	***-ap***	+179.35	***+ap***	-179.54	***-ap***	-177.08	***-ap***	-178.63	***-ap***	+176.74	***+ap***
$O_{23}C_{21}C_1C_2$	+122.19	***+ac***	+117.50	***+ac***	-102.46	***-ac***	+42.93	***+sc***	+169.59	***+ap***	-42.08	***-sc***
$O_{24}C_{22}C_2C_3$	+104.19	***+ac***	+61.13	***+sc***	-32.29	***-sc***	-28.55	***-sp***	+17.76	***+sp***	+36.38	***+sc***
$C_{25}O_{24}C_{22}C_2$	-82.31	***-sc***	-98.26	***-ac***	-85.55	***-sc***	-95.79	***-ac***	-99.69	***-ac***	-98.36	***-ac***
$H_{39}O_{23}C_{21}C_1$	+178.53	***+ap***	+178.08	***+ap***	-	-	-	-	-	-	-	-
$H_{45}O_{13}C_{11}N_9$	-	-	+156.77	***+ap***	+16.66	***+sp***	+118.04	***+ac***	+163.52	***+ap***	+133.79	***+ac***
$H_{44}O_{10}C_8N_6$	-	-	+172.25	***+ap***	+176.48	***+ap***	-8.77	***-sp***	+5.21	***+sp***	+168.58	***+ap***
$HN_6C_5S_4$	-	-	-	-	-	-	-	-	+138.34	***+ac***	-	-
$HN_9C_7C_5$	-	-	-	-	-	-	-178.97	***-ap***	-	-	-	-
$HN_{20}C_{19}C_{16}$	-	-	-	-	+167.04	***+ap***	-	-	-	-	-	-
* Conformational analyses using prefixes a = anti, s = syn, p = peri-planar, c = clinal, and + & - signs.[17]												

12. CONCLUSIONS

Cephapirin has been recognized as a broad spectrum antibiotic and capable of consuming the bacteria's oxygen and inhibited their growth. In practice, cephapirin may undergo tautomerism in aqueous solutions. The dipolar character of the molecule has been expected to influence selective penetration through the porin channels of the cell membrane. Computational methods are reliable to characterize the molecule because of their efficiency and accuracy with respect to the evaluation of a number of molecular properties. A suitable quantum chemical study is helpful to predict compound properties economically and to clarify some experimental phenomena insightfully. Austin Model-1 (AM1) is one of the semi-empirical methods and extensive simplification of Schrödinger's equation ($H\Psi = E\Psi$) to optimize molecules for calculation of various properties. In this respect, a brief review on the zwitterions of cephapirin tautomers has been taken up, along with the sequence of proton transfer to nitrogen atoms.

a) The spatial arrangement of atoms in the conformations of cephapirin (**1**), and its lactim (**2**), enol (**3**) and lactim-enol (**4**) are existed in *anti*- or *syn*- conformations. The net charges of nitrogen atoms in the order of $N_{18} < N_6 < N_9$ and the tautomeric equilibrium is increased in the order of $\log K_{T4} < \log K_{T1} < \log K_{T3} < \log K_{T2} < \log K_{T5}$ at the time of respective tautomeric conversion of **3** ↔ **4**, **1** ↔ **2**, **2** ↔ **4**, **1** ↔ **3** and **1** ↔ **4**. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **4 < 3 < 2 < 1**. Ionization potential (IP) is increased in the order of **4 < 2 < 3 < 1**. The dipole moment is increased in the order of molecules **3 < 2 < 1 < 4**. As per electron excitation energies (ΔE) (in eV), a large gap implies high stability and small gap implies low stability. The reactivity is decreased in the order of **4** > **3** > **2** > **1**.

b) The sequence of proton transfer for nitrogen atoms in cephapirin is decreasing in the order of $N_9 > N_6 > N_{18}$ for the formation of zwitterions, which are solvated to form hydrogen bonds with the polar solvents and it would affect the position of the equilibrium. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **3 < 4 < 2 < 1 < 5**. Ionization potential (IP) is increased in the order of **1 < 3 < 4 < 2 < 5**. The dipole moment is increased in the order of molecules **2 < 4 < 5 < 1 < 3**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **3** > **1** > **5** > **2** > **4**.

c) The sequence of proton transfer for nitrogen atoms in cephapirin-lactim is $N_9 > N_6 > N_{18}$ for the formation of zwitterions. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **4 < 3 < 5 < 2 < 1 <.6** Ionization potential (IP) is increased in the order of **6 < 3 < 5 < 2 < 1 < 4**. The dipole moment is increased in the order of molecules **2 < 1 < 4 < 5 < 6 < 3**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **3** > **5** > **4** > **6** > **2** > **1**.

d) The sequence of proton transfer for nitrogen atoms in cephapirin-enol is $N_9 > N_6 > N_{19}$ for the formation of zwitterions. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **5 < 3 < 4 < 2 < 1 < 6.** Ionization potential (IP) is increased in the order of **6 < 3 < 2 < 5 < 1 < 4**. The dipole moment is increased in the order of molecules **2 < 1 < 4 < 3 < 5 < 6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **1** > **3** > **6** > **5** > **2** > **4**.

e) The sequence of proton transfer for nitrogen atoms in cephapirin lactim-enol is $N_9 > N_{20} > N_6$ for the formation of zwitterions. According to the heat of formation (ΔH_f^o) data, the stability is increased in the order of **3 < 5 < 4 < 2 < 6 < 1.** Ionization potential (IP) is increased in the order of **6 < 3 < 4 < 2 < 1 < 5**. The dipole moment is increased in the order of molecules **1 < 5 < 2 < 4 < 3 < 6**. As per electron excitation energies (ΔE) (in eV), the reactivity is decreased in the order of **1 > 3 > 6 > 4 > 2 > 5**.

Further, the utility of theoretical predictions is important for evaluating the stability of zwitterions conformations and molecular deformations, which is highly dependent relative upon the polarity of the medium.

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