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Interaction of acetazolamide with antibiotic drugs

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Abstract : In the present study charge transfer interactions of an antihypertensive drug; acetazolamide with four antibiotics (amoxicillin, erythromycin, ofloxacin and ciprofloxacin) have been investigated at three physiological pH values and body temperature. The theoretical and experimental results revealed two antibiotics; erythromycin and amoxicillin to form charge transfer complexes of 1:1 stoichiometry with acetazolamide. The complexation ratio was proved by compelling evidences from UV-Vis spectroscopy and cyclic voltammetry. The theoretical findings supported the experimental results. The poor binding affinity of ofloxacin and ciprofloxacin suggested these as preferred antibiotics to be prescribed in combination with acetazolamide. The strong binding propensity of erythromycin and amoxicillin with acetazolamide suggested these antibiotics to be safely taken only after the digestion of Azm in stomach.

Keywords : Acetazolamide; Antibiotics; Drug-drug interaction; UV-Vis spectroscopy; Physiological conditions.

INTRODUCTION

In combination therapy, there is always a risk of drug interactions which can sometimes be strong enough to cause life-threatening injuries. Therefore, the clinicians find difficulty in the prescription of potentially interacting drugs[1]. The bioactivity, bioavailability, gastrointestinal absorption and dissolution of one drug can be altered by the simultaneous administration of the other[2-4]. For example the antibiotic availability is enhanced in the presence of hydrogen receptor antagonist[5]. Clinical studies reveal that tetracycline and fluoroquinolones are susceptible to clinically relevant drug-drug interactions with antacids[6,7]. Similarly, the co-prescription of antihypertensive and antibiotics has been found to complicate the treatment through adverse drug interactions. Anti-hypertensive efficiency of lovastatin[8], verapamil, diltiazem, nifedipine, amlodipine and felodipine[9] and activity of oral contraceptives[10,11] has been proved to be affected by antibiotics through clinical studies. Moreover, the direct charge transfer interactions between the drugs lower their effectiveness. As the activity of drugs in combination therapy can be modulated by drug interactions, so binding behavior of antihypertensive drug, acetazolamide (Azm) with antibiotics is the main focus of the current article.

Extensive literature is available on the study of drugs interaction within biological systems. Molecular docking[12], molecular mechanics[13,14] and molecular dynamic simulations[15] have been employed as successive

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computational tools to elucidate enzyme-drug interactions. Computer docking techniques are used for the establishment of action mechanism of drugs[16]. Computational tools are employed for the evaluation of electron donating and accepting capability of amino acids through HOMO and LUMO energies[15]. A variety of analytical techniques such as IR[17], NMR[13], UV-Vis spectrophotometry[18,19] and dissolution methods[18-20] have been reported for interactional studies of drugs. However, in case of charge transfer complexation between the drugs, UV-Vis spectroscopy and cyclic voltammetry are used as effective detecting tools[21-27]. Therefore, the detailed investigations of Azm- *antibiotics* binding were carried out at pH 3, 7 and 9.5 corresponding to pH of stomach[28], blood[29] and intestine[30] in order to get insights about the interactive pharmacokinetics of drugs during digestion and absorption in human body. These studies are vital for unfolding the mode of drug interactions and designing of more efficient drugs with positive/no/lesser side effects in combination therapy. Moreover, this research work is a useful guide for clinicians to prescribe the time difference that should be maintained between intakes of such drugs.

EXPERIMENTAL

Material and reagents

Commercial tablets of the drugs were used for the present investigations. 0.01M stock solutions of the compounds were prepared in dimethylsulfoxide after heating at 40 ºC for 2 hours and diluted with supporting electrolyte solutions (KCl /HCl (pH-3), Na_2CO_3/ $NaHCO_3$ (PH-9.5) and NaH_2PO_4/Na_2HPO_4 (pH-7)). All supporting electrolytes of 0.1 M strength were prepared using analytical grade reagents and doubly distilled water. The experiments were done at human body temperature.

DFT calculations

DFT calculations were performed on 6-311G*/ B3LYP basis set using Hyper-Chem Release 08 software. Geometric optimization was done at 6-31G*/ B3LYP. Frequency calculations at DFT level were done for individual and merged drugs with Azm.

UV-Vis spectroscopic and electrochemical measurements

Absorption spectra were recorded on Shimadzu 1601. Cyclic voltammetric investigations were done using conventional three electrode system of glassy carbon, saturated calomel and thin platinum wire acting as working, reference and counter electrodes respectively. Job's method of continuous variation[31] was employed to determine the stoichiometry of the complexes.

RESULTS AND DISCUSSION

Computational studies were done before experimental investigations. This helped in reducing the labor and cost required for the experimental studies of adverse drug combinations.

Theoretical calculations

Possible charge transfer complexation between the drugs was first predicted by theoretical calculations. On the basis of HOMO and LUMO energies, relative electron pair donating or accepting properties in co-administrated drugs were calculated. Scheme 1 demonstrates that energies of HOMO of Cipro and Oflox become less negative whereas, those of Amox and Erythro get more negative after combining with the LUMO of Azm. Thus, the outer electrons of Amox and Erythro get more tightly bound after complexation with Azm. Amox and Erythro are predicted to form stable 1:1 complex with Azm whereas, Cipro and Oflox are predicted to be inert to Azm.

The binding strength and free energy change of drug-drug complexation were calculated for different drug combinations at 310 K using protonated, neutral and deprotonated species corresponding to acidic, neutral and basic conditions (see TABLE 1). The negative ΔG of Azm-Amox and Azm-Erythro adducts revealed spontaneous complexation for protonated species and non-spontaneous in case of neutral and deprotonated species. The complexation of Azm-Oflox and Azm-Cipro adducts was also predicted to be non-spontaneous.

UV-Vis spectroscopic measurements

A band corresponding to $n \rightarrow \pi^*$ transition of Azm appeared at 267, 268 and 294 nm at pH-3, 7 and pH-9.5. In pH-3 Amox registered a band at 244 nm.

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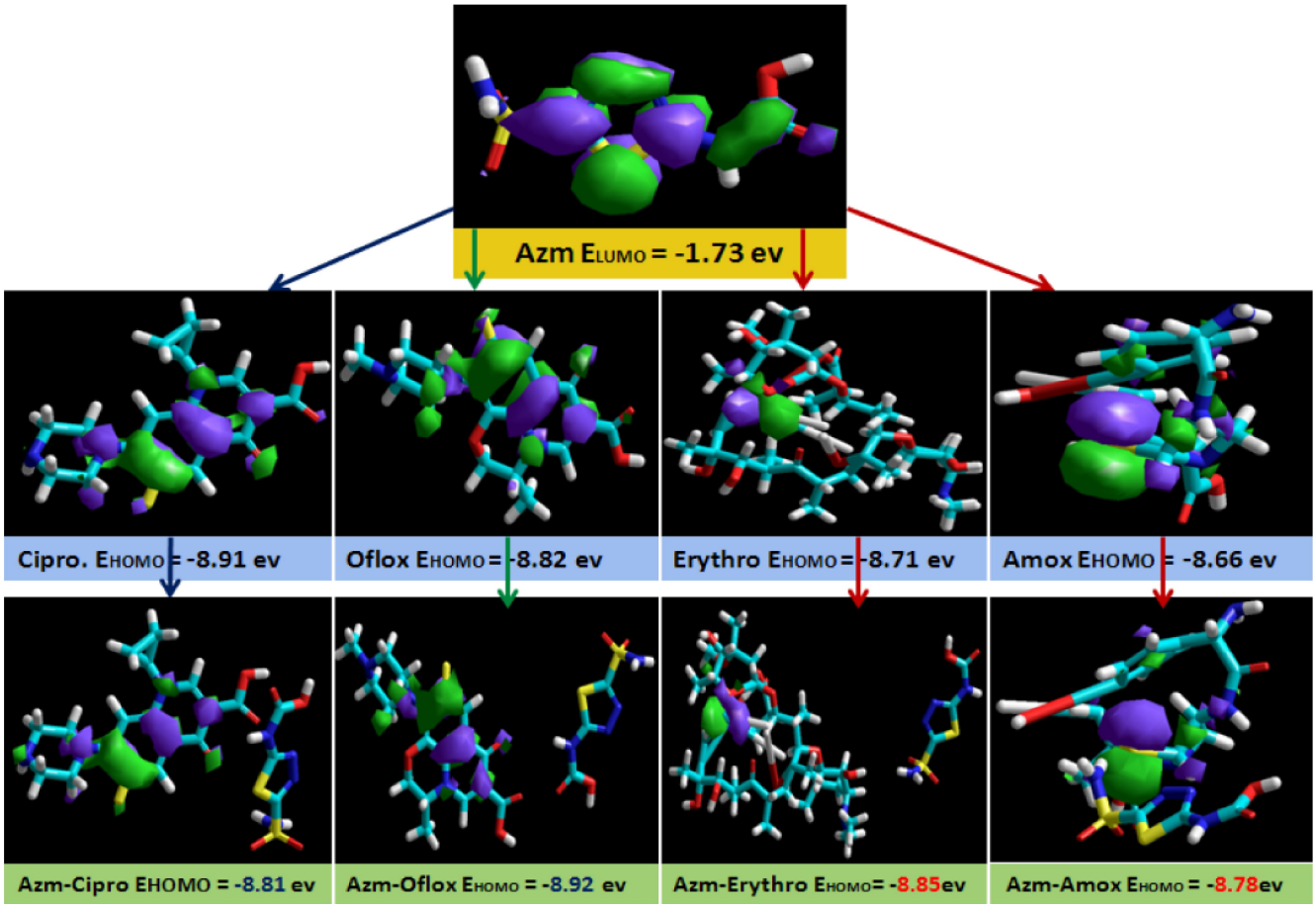

Scheme 1 : Molecular orbitals of Azm, antibiotics and their predicted complexes.

TABLE 1 : Formation constant and ΔG values of the complexes of antibiotics with antihypertensive acetazolamide at 310K as predicted by DFT.

Species	K (M^{-1})			ΔG ($kJmol^{-1}$)		
	Protonated specie	Neutral specie	Deprotonated specie	Protonated specie	Neutral specie	Deprotonated specie
Azm + Amox	124	0.39	0.024	-12.43	2.42	9.22
Azm + Cipro	7.3E-4	0.62	0.08	18.64	1.21	6.342
Azm + Erythro	74.22	0.26	0.71	-11.21	3.46	0.897
Azm + Oflox	8.4E-3	0.03	8.36E-3	12.31	8.93	12.33

Figure 1(a) shows the peak of Azm to intensify and shift bathochromically (from 268 nm to 285 nm) in the presence of Amox. These peculiar spectral characteristics are indicative of charge transfer complexation between the two drugs. Plot of absorbance *versus* mole fraction (Figure 1(b)) offers concrete evidence in support of 1:1 charge transfer complex formation between Azm and Amox. The increase in absorption accompanied with slight red shift was noticed for each incremental addition of Azm into excess of Amox and vice versa. This behavior offered another clue of charge transfer complex formation.

Amox was found to register two peaks at 240 and 271 nm at pH-7. Mixture of Azm and Amox exhibited two peaks at 268 and 242 nm, apparently with slight peak shift and hyperchromism in the range of 250-295 nm and hypochromism in the range of 235-250 nm Figure 2(a). Conservation of absorption behavior of both Azm and Amox in the mixture at pH-7 reveals that the observed hyperchromism and hypochromism are due to the additive absorption by both species and the overlapping spectrum is not due to charge transfer interactions. Similar results were obtained for Azm-Amox at pH-9.5 (Figure 2 (b)).

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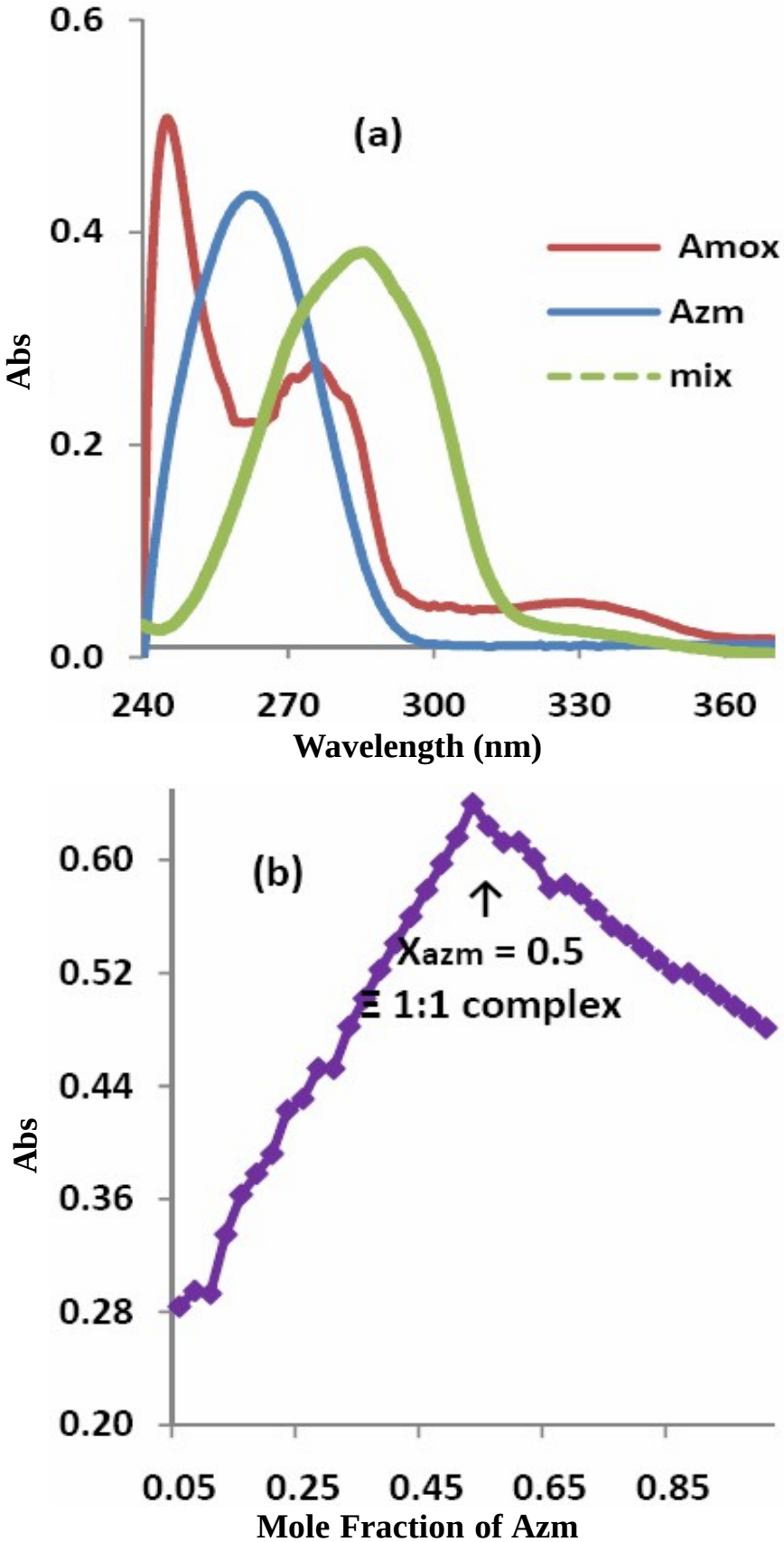

Figure 1 : UV-Vis spectra of Azm, Amox and their mixture at (a) pH-3, (b) Job's plot for Azm Amox.

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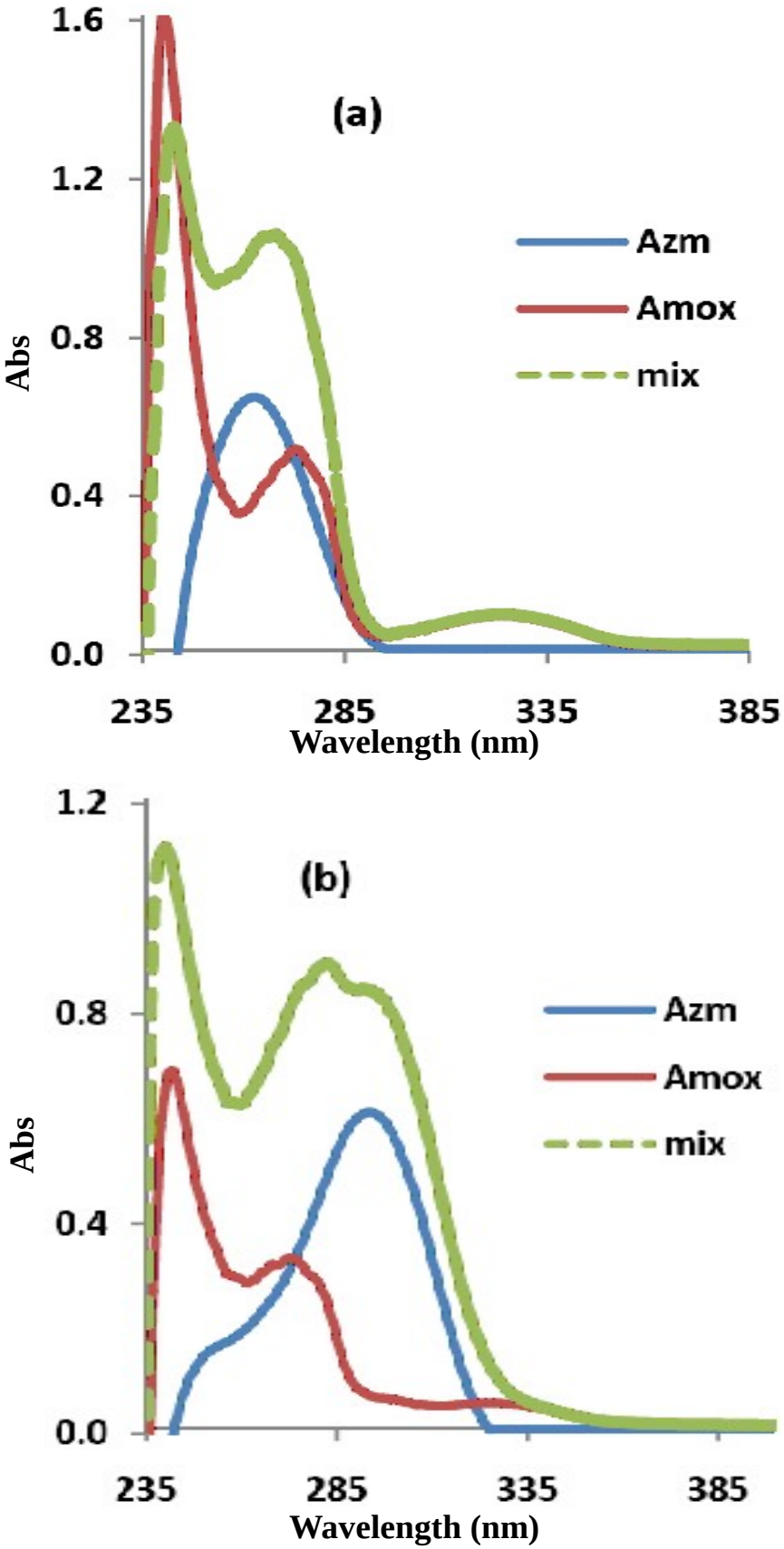

Figure 2 : UV-Vis spectra of Azm, Amox and their mixture at (a) pH-7 and (b) pH-9.5.

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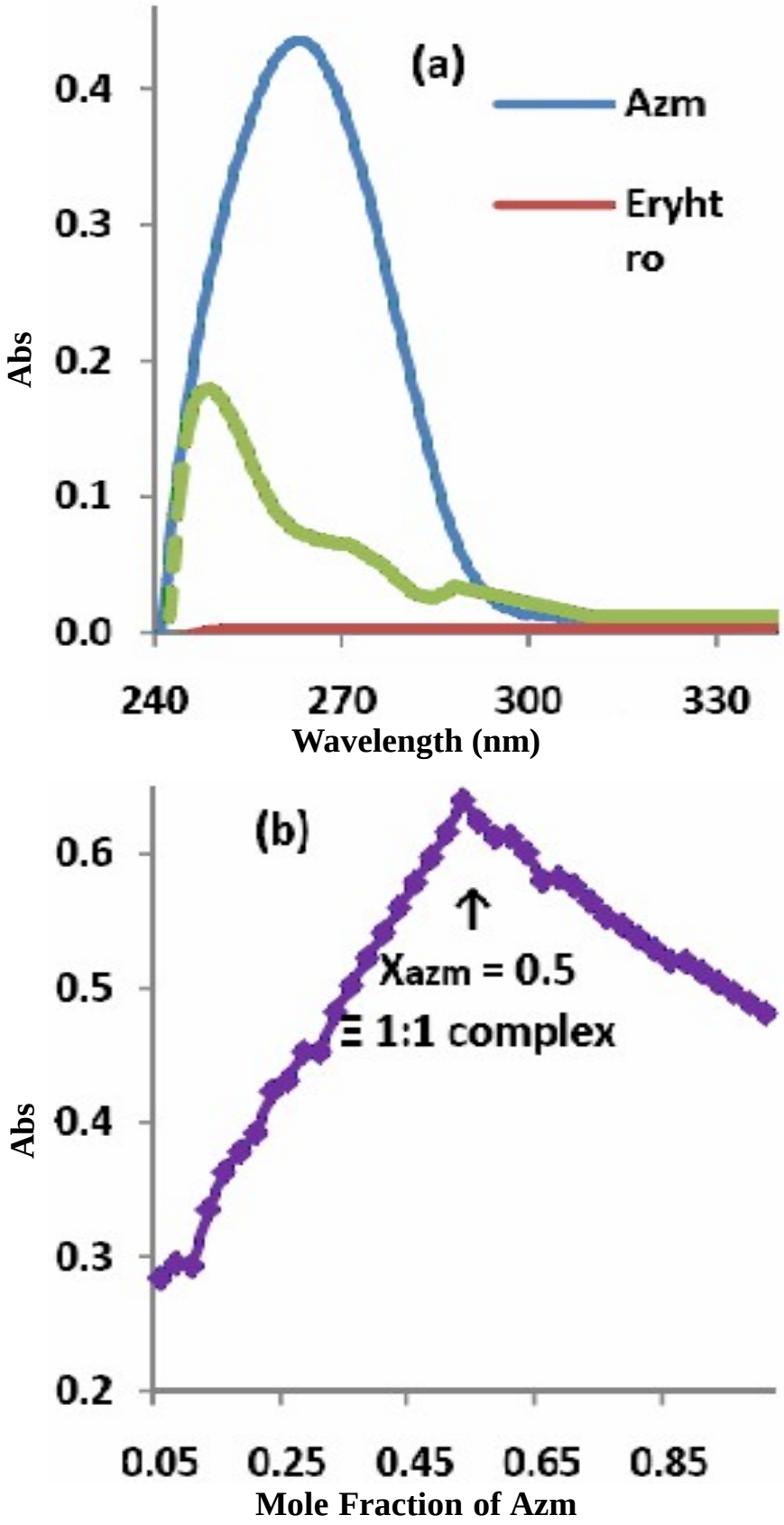

Figure 3 : (a) UV-Vis spectra of Azm, Erythro and their mixture at pH-3, (b) Job's plot for mix of Azm Erythro at pH-3 showing the formation of 1:1 complex.

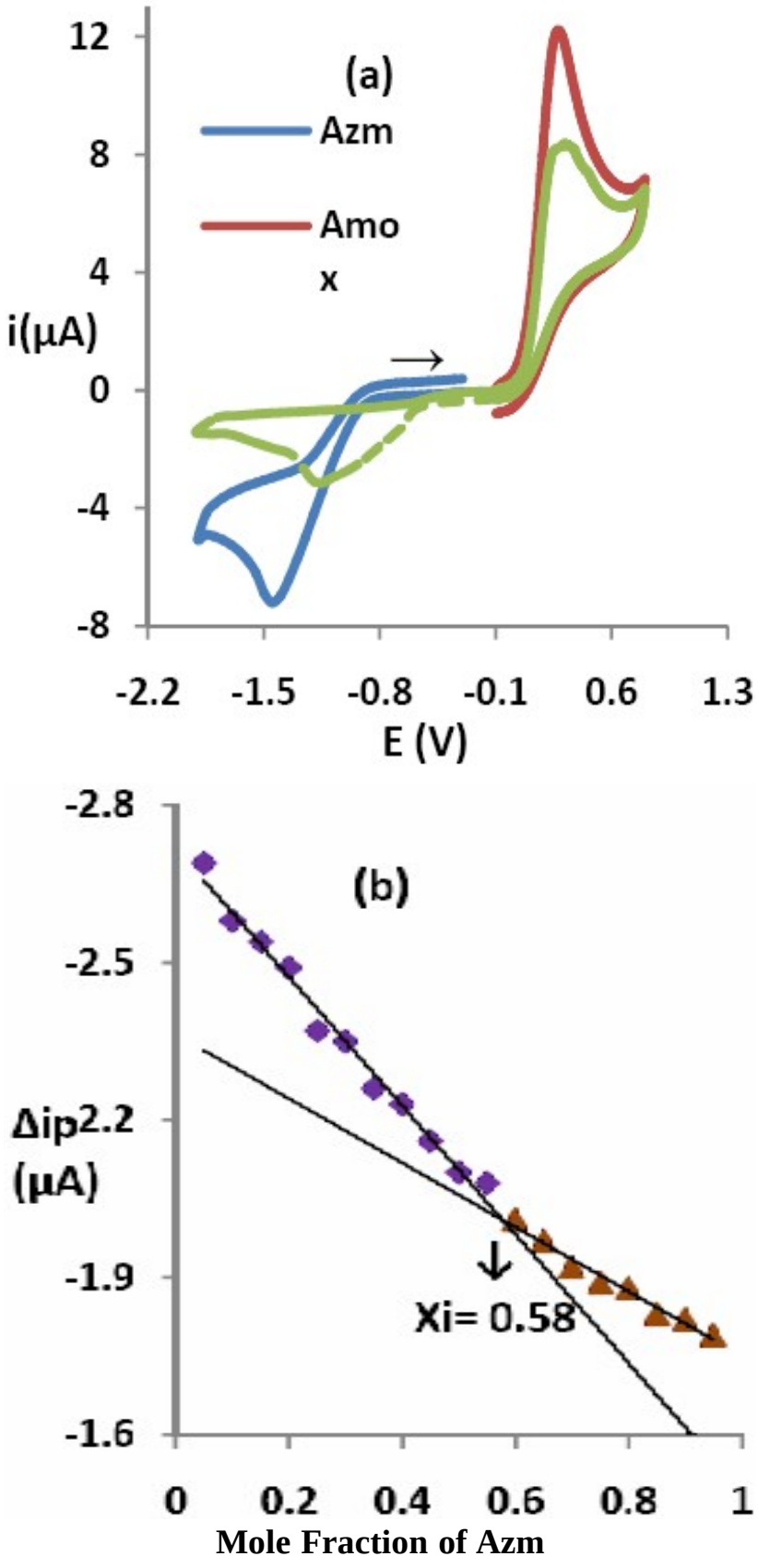

Figure 4 : Cyclic voltammograms of Azm, Amox and their mixture at (a) pH-3, (b) Job's type plot for Azm Amox mix at pH-3.

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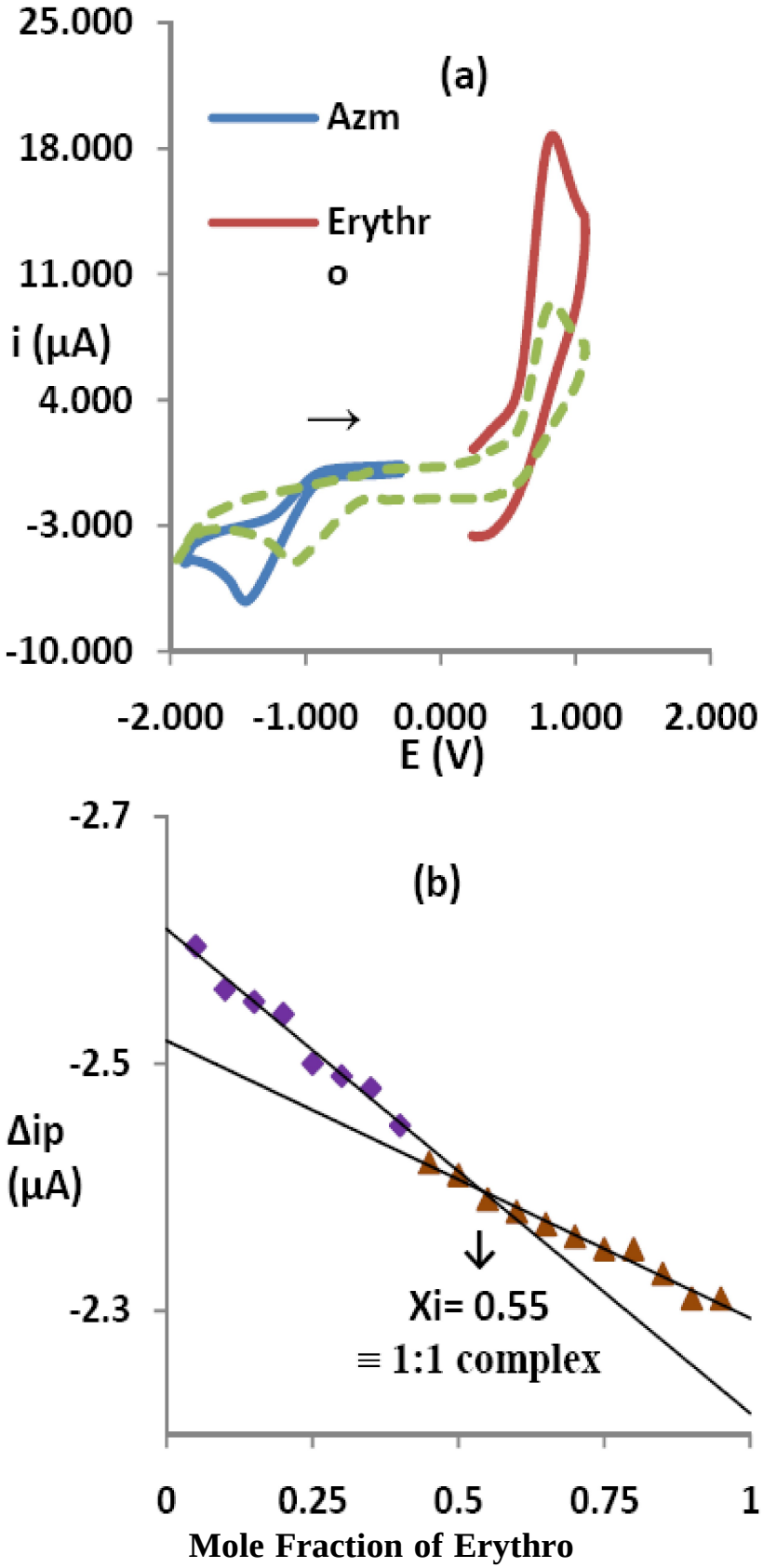

Figure 5 : (a) Cyclic voltammograms of Azm, Erythro and their mixture at pH-3, (b) Job's type plot for mix of Azm Erythro at pH-3.

TABLE 2 : Formation constants and ΔG values of the complexes of antibiotics with antihypertensive acetazolamide at 310±1.0 K as determined from UV-Vis spectroscopic data.

pH	Species	K_f (M^{-1})	ΔG (kJmol^{-1})
		Constant accepter, donor variable	Constant accepter, donor variable
3	Azm -Amox	1.1×10^{3}	-18.3
	Azm - Cipro	5.2×10^{-10}	55.2
	Azm -Erythro	5.0×10^{2}	-16.0
	Azm - Oflox	1.6×10^{-6}	34.4
7	Azm -Amox	2.5×10^{-3}	15.4
	Azm - Cipro	0.45	2.1
	Azm -Erythro	-	-
	Azm - Oflox	1.9×10^{-2}	10.2
9.5	Azm -Amox	6.6×10^{-3}	12.9
	Azm - Cipro	2.0×10^{-2}	10.1
	Azm -Erythro	-	-
	Azm - Oflox	3.0×10^{-5}	26.6

Erythromycin has been reported to give no UV-Vis absorption band at pH 3, 7 and 9.5[32]. Absorption band of 0.1 mM Azm gets hypsochromically shifted from 267 to 255 nm (Figure 3(a)) in the presence of 0.1 mM solution of Erythromycin in pH-3. The hypsochromic peak shifting of Azm by incremental addition of either Erythro to the excess of Azm or addition of Azm to the excess of Erythro is indication of Azm-Erythro interaction at pH-3. Job's plot shown in Figure 3(b) depicts the formation of 1:1 complex between erythromycin and Azm. Similar results were obtained for Azm-Erythro pair at either pH-7 or 9.5. Spectral analysis of Azm in the presence of either Cipro or Oflox at pH-3, 7 and 9.5 revealed inert behavior of drugs towards Azm.

The formation constant was evaluated from the plot of absorption intensity as a function of concentration of either component using the following modified form of Benesi-Hildebrand equation[33].

TABLE 3 : Characteristic, kinetic and binding parameters of Azm, antibiotics and Azm-antibiotics mixtures evaluated from cyclic voltammetric data at 310±1K.

Species		E_p (V)	Kinetic parameters				Binding parameters	
			$D_f \times 10^6$ (cm^2/s)	$D_b \times 10^6$ (cm^2/s)	$k_{(f)} \times 10^4$ (cm/s)	$k_{(b)} \times 10^4$ (cm/s)	$K_f \times 10^3$ (M^{-1})	?G (kJmol^{-1})
pH-3	Azm	-1.448	1.823	-	2.313	-	-	-
	Amox	0.272	0.532	0.823	3.421	1.045	8.3E6	-17.29
	Erythro	1.442	1.026	0.080	1.542	0.247	5.2E5	-16.01
	Cipro	0.825	0.155	1.673	3.874	1.923	5.8	12.1
	Oflox	0.213	0.098	1.712	0.768	2.424	0.1	21.1
pH-7	Azm	-1.383	1.681	-	2.152	-	-	-
	Amox	0.286	0.213	1.264	2.816	1.846	4.1	13.1
	Erythro	1.475	0.986	1.236	1.345	1.987	4.0	13.0
	Cipro	0.804	0.099	1.437	1.943	2.124	600	21.5
	Oflox	0.247	0.077	1.432	0.691	2.213	11	12.2
pH-9.5	Azm	-1.284	1.192	-	1.174	-	-	-
	Amox	0.479	0.413	0.867	1.297	1.098	1.5	15.6
	Erythro	1.606	0.776	0.973	0.675	1.093	5E-8	61.2
	Cipro	0.897	0.125	0.996	2.962	1.116	4	11.5
	Oflox	0.298	0.094	1.042	0.593	1.097	0.3	25.1

$$\frac{[D_1]}{Abs} = \frac{1}{K_{AD}\,\varepsilon_{AD}} \cdot \frac{1}{[D_2]} + \frac{1}{\varepsilon_{AD}} \quad \textbf{(1)}$$

Or alternate equation;

$$\frac{[D_2]}{Abs} = \frac{1}{K_{AD}\,\varepsilon_{AD}} \cdot \frac{1}{[D_1]} + \frac{1}{\varepsilon_{AD}} \quad \textbf{(2)}$$

Large values of K_f and negative ΔG (TABLE 2) for Azm-Amox mixture and Azm-Erythro mixture indicate their complex formation to be spontaneous at pH-3 and non-spontaneous at pH-7 and 9.5. An examination of TABLE 2 further reveals the inability of Cipro and Oflox to interact with Azm.

Electrochemical measurements

In a medium of pH-3, a cathodic peak at -1.45 V

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and anodic peak at 0.27 V appeared in the cyclic voltammograms of Azm and Amox respectively (Figure 4a). In the presence of Amox the cathodic peak potential of Azm shifted from -1.45 V to -1.19 V with decrease in current intensity. This behavior is attributed to the charge transfer complexation between Azm and Amox. A significant decay in the peak current of Amox indicates that some of its amount is being consumed in complexing with Azm. The bulky complex is suggested to diffuse slowly towards the electrode surface as compared to free drugs. In Job's type plot (Figure 4b) between current decrement and mole fraction two intersecting straight lines were obtained. The point of intersection represented the formation of 1:1 Azm-Amox complex.

Single irreversible anodic peak in the cyclic voltammogram of erythromycin obtained in a medium of pH-3 was found to change its location from -1.45 V -1.10 V accompanied with the decrease in current intensity in the presence of Azm (Figure 5a). The current-mole fraction plot (Figure 5b) of two linear segments with point of intersection corresponding to 0.5 mole fraction of Azm supported the spectrophotometrically determined Azm-Eryhthro complex formation of 1:1 stoichiometry.

Similar treatment of Azm-Amox mixture and Azm-Erythro mixture at pH-7 and 9.5 resulted in reproduction of individual independent cyclic voltammetric behavior thus indicating no charge transfer interaction. Similar experiments performed for Azm-Cipro and Azm-Oflox mixtures at pH-3, 7 and 9.5 also revealed no interactions.

Formation constants were evaluated from voltammetric titrations of Azm with antibiotics and vice versa by employing the following equation

$$\frac{1}{[Drug]} = \left[\frac{K_f\,(1-A)}{\left\{1-\frac{I}{I_0}\right\}}\right] - K_f \qquad (3)$$

The high K_f and negative ΔG value of Azm-Amox and Azm-Erythro mixture at pH-3 show the spontaneity of their complex formation (TABLE 3). These electrochemical results complement the spectroscopic findings.

The kinetic parameters like diffusion coefficient (D_o) and heterogeneous electron transfer rate constant (k°_{sh}) were evaluated by using the following equations

$$I_p = 3.36 \times 10^6\, n\,(\alpha n_a)^{\frac{1}{2}} A\, C_o\, D_O^{\frac{1}{2}}\, \nu^{\frac{1}{2}} \qquad (4)$$

$$k^{\circ}_{sh} = -0.48\alpha + 0.52 + log\left(\frac{n\alpha \upsilon_c D_O}{2.303RT}\right) \qquad (5)$$

An examination of k°_{sh} and D_o values listed in TABLE 3, reveals that both parameters of Azm are decreased only slightly by non-interacting antibiotics e.g. either Amox or Erythro at pH-7 and 9.5 and either Cipro or Oflox at pH-3, 7 and 9.5 due to electrophoresis. On the other hand kinetic parameters for Azm-Amox mixture and Azm-Erythro mixture at pH-3 are much less than those of pure Azm. The rationale behind this is the formation of complexes in both cases which are electroreduced at the electrode surface and thus cause shift in cathodic peak of Azm but not in the oxidation peak of either Amox or Erythro. Complex being more bulky diffuses slowly than pure drugs. This reduces electron transfer rate constant and diffusion coefficient of the resultant complex.

CONCLUSION

Four antibiotics; amoxicillin, erythromycin, ofloxacin and ciprofloxacin were first investigated computationally to predict the possibility of their charge transfer interaction with antihypertensive acetazolamide (Azm). Orbital energies of HOMO and LUMO were used as a criterion to predict the possibility of complex formation between the donor and acceptor. Orbital energies predicted that all the four antibiotics are able to donate electrons to the LUMO of acetazolamide to form charge transfer complexes. But stabilization of orbitals and free energy change from DFT calculations predicted that out of the studied four antibiotics only two (Amox and Erythro) are able to form stable charge transfer complexes with antihypertensive acetazolamide.

The changes in electronic absorption and cyclic voltammetric behavior of Azm in the presence of antibiotics were successfully exploited for the verification of computational predictions. The obtained formation constants and free energy changes reflected the capability of Amox and Erythro to form charge transfer complexes with Azm. Acidic pH facilitated the charge transfer complexation more than either basic or neutral conditions. The poor binding affinity of Oflox and Cipro suggested these as preferred antibiotics to be prescribed

in combination with acetazolamide. However, the interaction of Amox and Erythro with acetazolamide under acidic conditions i.e., at the pH of stomach prohibited their combined prescription with Azm. These drugs can be safely taken only after the digestion of Azm in stomach.

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REFERENCES

[1] P.E.Gronroos, K.M.Irjala, R.K.Huupponen, H.Scheinin, J.Forsstrom, J.J.Forsstrom; Eur.J.Clin. Pharmacol., **53**, 13-17 **(1997)**.
[2] B.Parameshappa, V.N.Rao, S.T.Gouda, S.Sen, R.Chakraborty, M.A.Basha, A.S.Reddy, S.M.S.Kumar; Annals of Biological Research, **1**, 35-40 **(2010)**.
[3] N.Sultana, T.Mubeen, M.S.Arayne; Pak.J.Pharm. Sci., **14**, 1-8 **(2001)**.
[4] F.Hussain, S.M.Arayne, N.Sultana; Pak.J.Pharm. Sci., **19**, 16-21 **(2006)**.
[5] M.S.Arayne, N.Sultana, M.Afzal, A.Z.Mirza; J.Chem.Soc.Pak., **30**, 734-739 **(2008)**.
[6] R.Ogawa, H.Echizen; Drugs, **71**, 1839-1864 **(2011)**.
[7] P.Perite, T.Mazzei, E.Mini, A.Novelli; Clinical Pharmacokinetics, **23**, 106-131 **(1992)**.
[8] Beverly A.Kroner; Diabetes Spectrum, **15**, 249-255 **(2002)**.
[9] N.Blazek; Antibiotic, Clinical Advisor, **(2011)**.
[10] S.S.DeRossi, E.V.Hersh; Dent.Clin.N.Am., **46**, 653-664 **(2002)**.
[11] D.J.Back, S.F.M.Grimmer, M.L'e Orme, Christine Proudlove, R.D.Mann, A.M.Breckenridge; Br.J. Clin.Pharmac., **25**, 527-532 **(1988)**.
[12] J.Mannu, P.Jenardhanan, P.P.Mathur; J.Mol.Model., **17**, 1847-1854 **(2011)**.
[13] T.M.El-Gogary; Spectrochimica Acta Part A, **59**, 1009-1015 **(2003)**.
[14] J.Zhou, B.Coate, Y.He; Empirical power computation using SAS for Schuirmann's two one-sided tests procedure in clinical pharmacokinetic drug-drug interaction studies, SUGI 29 Proceedings, 154_29 **(2004)**.
[15] W.Wang, P.A.Kollman; PNAS, **98**, 14937-14942 **(2001)**.
[16] Z.Amtul, C.Follmer, S.Mahboob, A.U.Rahman, M.Mazhar, K.M.Khan, R.A.Siddiqui, S.Muhammad, S.A.Kazmi, M.I.Choudhary; Biochem. & Biophy.Research Comm., **356**, 457-463 **(2007)**.
[17] A.I.Darwish; Analytica Chimica Acta, **549**, 212-220 **(2005)**.
[18] F.Hussain, S.M.Arayne, N.Sultana; Pak.J.Pharm. Sci., **19**, 16-21 **(2006)**.
[19] M.S.Arayne, N.Sultana, M.H.Zuberi; J.Chil.Chem. Soc., **2**, 206-211 **(2010)**.
[20] N.Sultana, T.Mubeen, S.M.Arayne; Pak.J.Pharm. Sci., **14**, 1-8 **(2001)**.
[21] A.A.Ibrahim; African J.Pure & Appl.Chem., **5**, 507-514 **(2011)**.
[22] S.Mizyed, H.Tarabsheh, D.Marji; Jord.J.Chem., **2**, 145-153 **(2007)**.
[23] A.G.Saleh, F.H.Askal, A.I.Darwish, A.A.El-Shorbagi; Ana.Science, **19**, 281-287 **(2003)**.
[24] A.A.Ibrahem; Asian Transactions on Basic and Applied Sciences, **1**, 42-52 **(2012)**.
[25] S.Cakir, E.Bicer; Turk.J.Chem., **31**, 223-231 **(2007)**.
[26] H.Tang, A.Kitani, M.Shiotani; J.App.Electrochem., **26**, 36-44 **(1996)**.
[27] A.S.Aljabir, E.Nour; Spectrochimica Acta Part A, **70**, 997-1000 **(2008)**.
[28] P.N.Daykin; Orthomolecular Psychiatry, **5**, 212-214 **(1976)**.
[29] M.Kincl, M.Meleh, M.Veber; Acta Chim.Slov., **51**, 409-425 **(2004)**.
[30] M.N.Martinez, L.A.Gordon; J.Clin. Pharmacol., **42**, 620-643 **(2002)**.
[31] K.C.Ingham; Analytical Biochem., **68**, 660-663 **(1975)**.
[32] M.A.El-Bondkly, I.H.Abd-Alla, M.Shaaban, A.K.Shaaban; Indian J.Pharm.Sci., **70**, 310-319 **(2008)**.
[33] S.Li Yun, Q.L.Guo, W.Yuan; Bull.Chem.Soc. Ethiop., **24**, 21-30 **(2010)**.