Journal of Contemporary Research in Chemistry (2017) 2(1): 41-52

Investigation of interactions of acetzolamide with tricil at pH = 3.0, pH = 7.0 and pH = 9.5 through UV-Vis spectroscopy and cyclic voltammetry

Erum Jabeen*, Romana Qureshi
Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan

Abstract

The interaction of antihypertensive drug acetazolamide (Azm) with antacid drug tricil was investigated at pH = 3.0, pH = 7.0 and pH = 9.5 with the objective of monitoring their interactive pharmacokinetics during digestion and absorption in human body. The variation in absorbance and current of one drug caused by the addition of another drug revealed that tricil interacted with Azm in both acidic (pH = 3.0) and basic conditions (pH = 9.5). But these two drugs did not interact with each other at neutral pH (pH = 7.0). Further, in acidic conditions Azm-tricil formed 1:1 complex while these formed 1:2 complex at pH = 9.5 (basic conditions).

Keywords: *Stoichiometry, Benesi-Hildebrand equation, Interactions, Formation Constants*

Corresponding author E-mail: erum.jabeen@aiou.edu.pk (Eum Jabeen)

Introduction

Combination therapy is the term used to define the simultaneous treatment of more than one disease. Some diseases such as hypertension, diabetes, hepatitis etc. (Fereidoonnezhad et al., 2017) require long term treatment while other are usually only short treatment such as inflammation, infections etc. Therefore, it may happen that during the treatment of long term illness, short term therapies would be required. Further, treatment of some illnesses consists of combination therapy to overcome their side effects for example anti-rheumatic drugs need PPI's (proton pump inhibitors) (Henriksson et al., 2014). The clinicians needs to be aware of potentially interacting drug combinations before writing prescription as drug-drug interactions can lead to serious threats to human life (Gandhi et al., 2010). More often, however, they can be the cause of slow recoveries or these can induce slight symptoms. In severe cases, there are chances of potential injury instead of recovery. The bioactivity (Parameshappa et al., 2010), bioavailability (Srinivasan, (2001), gastro-intestinal absorption and dissolution of the drugs (Hussain et al., 2006) can be hindered by simultaneous injection of potentially adduct forming drugs.

Antacid is the term which defines the cure of acidity, heartburning and ulceration etc. (Kreuteret al., 2016). It has been reported that antacids have complicated the treatment of hypertension when Captoril (Li et al., 2010), Lisinopril (Teleb et al., 2004), Indapamide, Chalorothiazide, Chlorothalidone and hydrochlorothalizide (Bhattacharya et al., 2001) are used as antihypertensive agent. Significant drug interactions with antacids have been reported for certain members of the Quinolone, non-steroidal anti-inflammatory drug (NSAID) and cephalosporin (Sadowski et al., 1994). Adduct forming antacid-drug interactions can lead to alteration of their gastrointestinal motility or changes in urinary or gastric acidity theoretically. Direct adsorption of a drug over other can also reduce its bio-availability or drug action. The bioavailabilities of Cimetidine and Ranitidine which are the H_2-receptor antagonist have been reported to be reduced by antacids (Mihaly et al., 1982; Grasela et al., 1989). The polyvalent metal cations present in formulations of antacid

can be metal chelating source for ligand binding agents. This can result in formation of chelate complexes of one drug with other substantially reducing their bioavailability. Therefore, in the present work, the investigations of binding of an antihypertensive drug acetzolamide with antacid tricil were done through UV-Vis spectroscopy and cyclic voltammetry (Jabeen et al., 2013). The investigatory pHs were acidic pH = 3, neutral pH = 7 and basic pH = 9.5 which may persist in different parts of gastrointestinal track (Marieb et al., 2010, Marques et al., 2011, Uhde et al., 2016).

Materials and methods

Commercial tablets of acetazolamide and Tricil were used for the present investigations. The components of buffer solutions KCl/HCl (pH = 3.0), NaH_2PO_4/Na_2HPO_4 (pH = 7.0) and $Na_2CO_3/NaHCO_3$ (pH = 9.5) (Jabeen et al., 2013; Blachier et al., 2010) were obtained from Sigma Aldrich. Distilled water was used for dilution. Stock solutions of analytes were prepared in 1% DMSO in appropriate aqueous buffer solutions in DMSO.

The values of absorbance obtained from absorptive titration were plotted through Benesi-Hildebrand equation (Li et al., 2010) to evaluate binding constants.

$$\frac{[A_0]}{Abs} = \frac{1}{K_f \varepsilon_{comp}} . \frac{1}{[D_0]} + \frac{1}{\varepsilon_{comp}} \quad (1)$$

Where;

$[A_0]$ = Initial concentration of Accepter = Acetazolamide

$[D_0]$ = Initial concentration Donor = Tricil

Abs = Absorbance at peak position

K_f = Formation constant of complex

ε_{comp} = Molar Absorbance of complex formed

From Benesi-Hildebrand equation, ε_{comp} can be obtained from intercept while ratio of intercept to slope is equal to K_f for drug-drug interactions.

The absorbance of mixture of the solution was monitored as a function of mole fraction of Azm. Cyclic voltammetric investigations were performed using conventional three electrode system consisting of glassy carbon, SCE (saturated calomel electrode) and thin Platinum wire as working, reference and counter electrode, respectively. The electrode was polished for about 1 min with emery paper of 300 grit then washed thoroughly with distilled water and mixture of DMSO and buffer solution prior to each recording. The de-oxygenation of the solutions was done by purging all solutions with high purity argon gas before every electrochemical experiment. Every sample was kept in electrochemical cell for 2-3 min in order to attain temperature of 310 K (corresponding to body temperature). Cyclic voltammetry of pure drugs followed by the voltammetric titration of constant Azm with Tricil was carried out at pH = 3.0, pH = 7.0 and pH = 9.5. Cyclic voltammograms of pure analyte of drugs (1 mM) were recorded at scan rates varying from 0.001 Vs^{-1} to 1.5 Vs^{-1} to calculate heterogeneous electron transfer rate constant and diffusion coefficient.

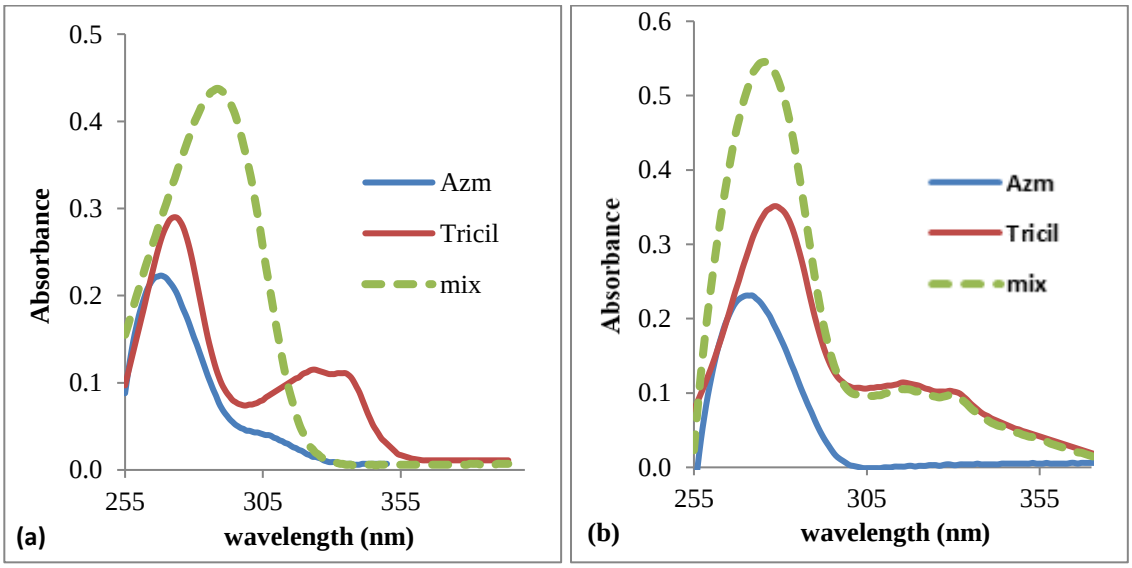
In Jobs method the current of mixtures of the solutions were monitored as a function of mole fraction of either component to find the stoichiometry of resulting species according to reported method (Jabeen et al., 2013).

Results and Discussion

UV-Vis Spectroscopic Measurements

It is well known from literature (Kalanur et al., 2009) that the absorbance band of drug shifted when drugs interacts with DNA. If the drug interacts with another drug, the shift in the UV-Vis band of drugs upon addition of other drugs can be used as a tool for elucidation of drug-drug interaction.

By the addition of 1 mM antacid into 0.1 mM solution of Azm at pH = 3.0 hyperchromism along with red shift from 267 nm of Azm to 291 nm of adduct was observed which clearly indicated the formation of complex between Azm and tricil (Fig.1 (a)). Incremental addition of antacid in Azm resulted in sequential red shift along with hyperchromacity which when exploited through Benesi-Hildebrand equation resulted in a straight line (Fig. 2). This confirmed interaction of Azm with tricil at pH = 3.0.



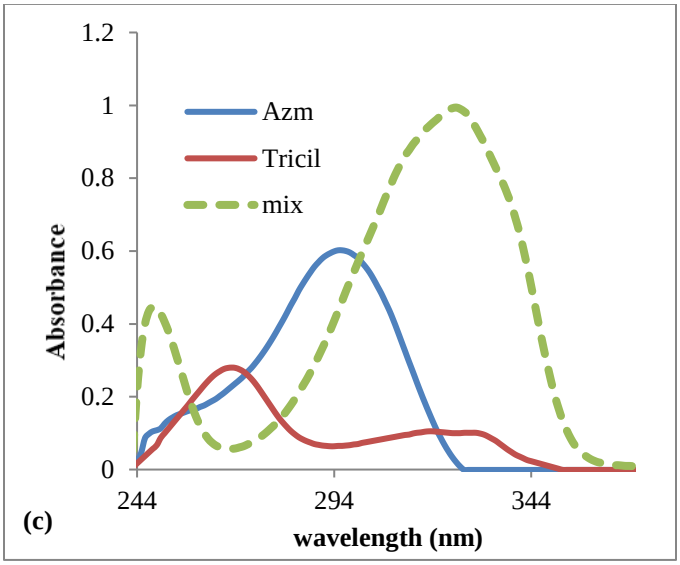


Fig. 1 Spectra of Azm, tricil and their mixture at (a) pH = 3.0, (b) pH = 7.0, (c) pH = 9.5.

λ_{max} of Azm was found to be 268 nm and that of tricil was found to be 278 nm, 312 nm and 327 nm at pH = 7.0. Addition of 1 mM tricil in 0.1 mM solution of Azm at pH = 7.0 caused hyperchromism with a peak at 272 nm (at average of original peaks) below 300 nm while peaks of tricil were reproduced in the range 300 nm to 400 nm (Fig.1 (b)). Reproduction of tricil peaks above 300 nm indicated no interaction. In order to clarify the observation differential method was conducted. In differential method the Azm-tricil mixture was placed in sample cuvit while the Azm was placed in reference cuvette as well. The concentration of Azm was same in reference as well as in sample cuvette. When Azm-tricil did not interact to generate some new specie, the addition of Azm in reference cuvette resulted in exactly reproduction of tricil spectra by subtracting Azm absorption form the mixture throughout λ range. The experiment was repeated to reproduce Azm spectrum form Azm-tricil mixture by keeping tricil in reference cuvette, In differential method, original spectra were reproduced confirming no interaction between the drugs at pH = 7.0.

Azm has an absorption band at 293 nm while tricil has two maxima at 269 nm and 334 nm in UV-Visible absorption spectrum at pH = 9.5. When 0.1 mM solution of Azm was mixed with 1 mM solution of tricil, a peak shift was observed and two new peaks at 246 nm and 329 nm were obtained instead of peaks corresponding to either of drug (Fig.1 (c)). This implies strong tricil interaction with acetzolamide at pH = 9.5. The incremental addition of tricil to Azm resulted in straight line Fig. 2 in accordance with Benesi-Hildebrand equation.

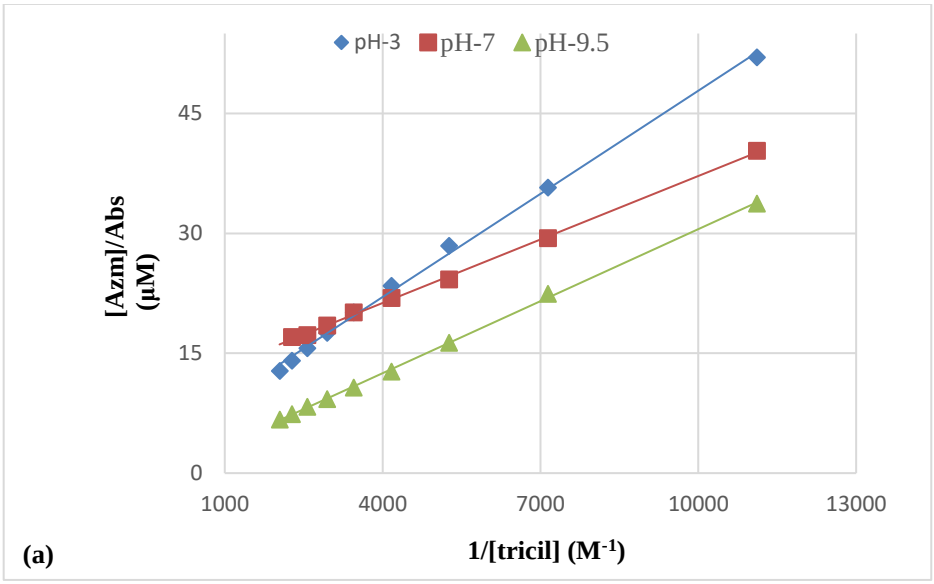


Fig. 2 Benesi-Hildebrand plot of constant Azm ([Azm] = 0.01 mM) and varied Tricil concentrations at pH = 3.0. pH = 7.0 and pH = 9.5.

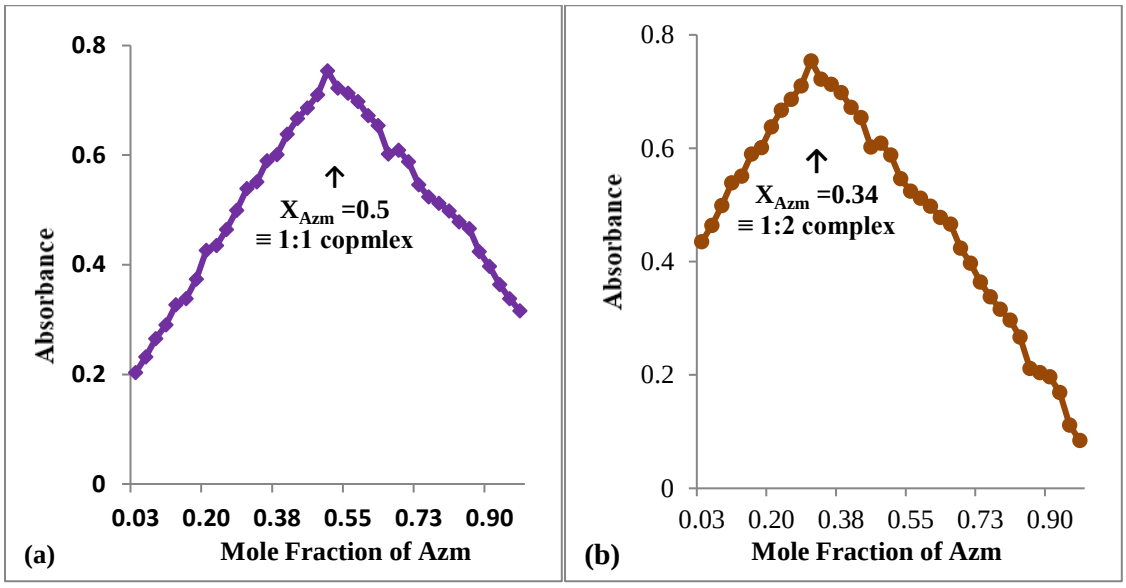


Fig. 3 Job plots for Azm-tricil mixtures of different mole fractions at (a) pH = 3.0 and (b) pH = 9.5.

Jobs method (Fig. 3) was applied to find the composition of resulting species. In Jobs method the absorption of mixture of interacting compounds at the λ_{max} of adduct/complex is plotted as function of mole fraction and a maxima is obtained at the stoichiometric mole fraction. Spectrophotometric data confirmed the formation of 1:1 charge transfer complex between Azm and tricil at pH 3 and formation of 1:2 complexes at pH = 9.5.

This shows the stoichiometry of the Azm-tricil depends on the pH of the solution. The stability constant was calculated using Benesi-Hildebrand equation where the linear [azm]/Abs. Vs 1/ [tricil] relationship was exhibited at every pH = 7 but the slope to intercept ratio significantly high at pH = 3.0 and pH = 9.5. Further, at pH = 7.0, ΔG = -

$RTlnK_f$ was found to be positive owing to the non-spontaneity of process at pH = 7.0 whereas it was negative revealing spontaneous adduct formation at pH = 3.0 and pH = 9.5 (Table 1). This reveals that certain ion formations may be involved in adduct formation that is why adduct is not being formed in neutral pH condition (pH =7.0).

Table 1

Formation constants and ΔG for Azm-Tricil interaction 310±1.0 K.

pH	Constant Accepter, Donor variable		Constant Donor, Accepter variable	
	K_f (M^{-1})	ΔG (kJ/mol)	K_f (M^{-1})	ΔG (kJ/mol)
3	666	-16.76	658	-16.24
7	0.410	2.297	0.33	2.88
9.5	1650	-19.09	1660	-19.12

Electrochemical Measurements

The variation of cyclic voltammograms of one drug by the addition of the other drug can be used for electrochemical investigation of drug-drug interactions. If the an electroactive drug has interacted with some other drug there will be a shift in peak potential or change in peak current or both which can be exploited to investigate complex formation drug-drug interactions.

Cyclic voltammogram of Azm shows a cathodic peak at -1.448V at pH = 3.0, -1.383 at pH = 7.0 and -1.284V at pH = 9.5 whereas the cyclic voltammogram of tricil also has cathodic peaks at -1.189V at pH = 3.0, -1.251V at pH = 7.0 and -1.272 V at pH = 9.5 (Fig. 4).

A clear peak shifting was observed at pH = 3.0 and pH = 9.5 by mixing of equimolar solutions of acetzolamide and tricil. Peak of acetzolamide shifted from -1.448V to -0.909V at pH = 3.0 (Fig. 4 (a)) and from -1.284V to -1.242V at pH = 9.5 (Fig.4 (b)). Shifting of peak and the peak current is not equal at both pH showing that the species formed at both pHs are not same. There was no such current decrease at pH = 7.0 only cumulative current resulting from the simultaneous oxidation of more than one compound was observed (Fig. 4 (c)).

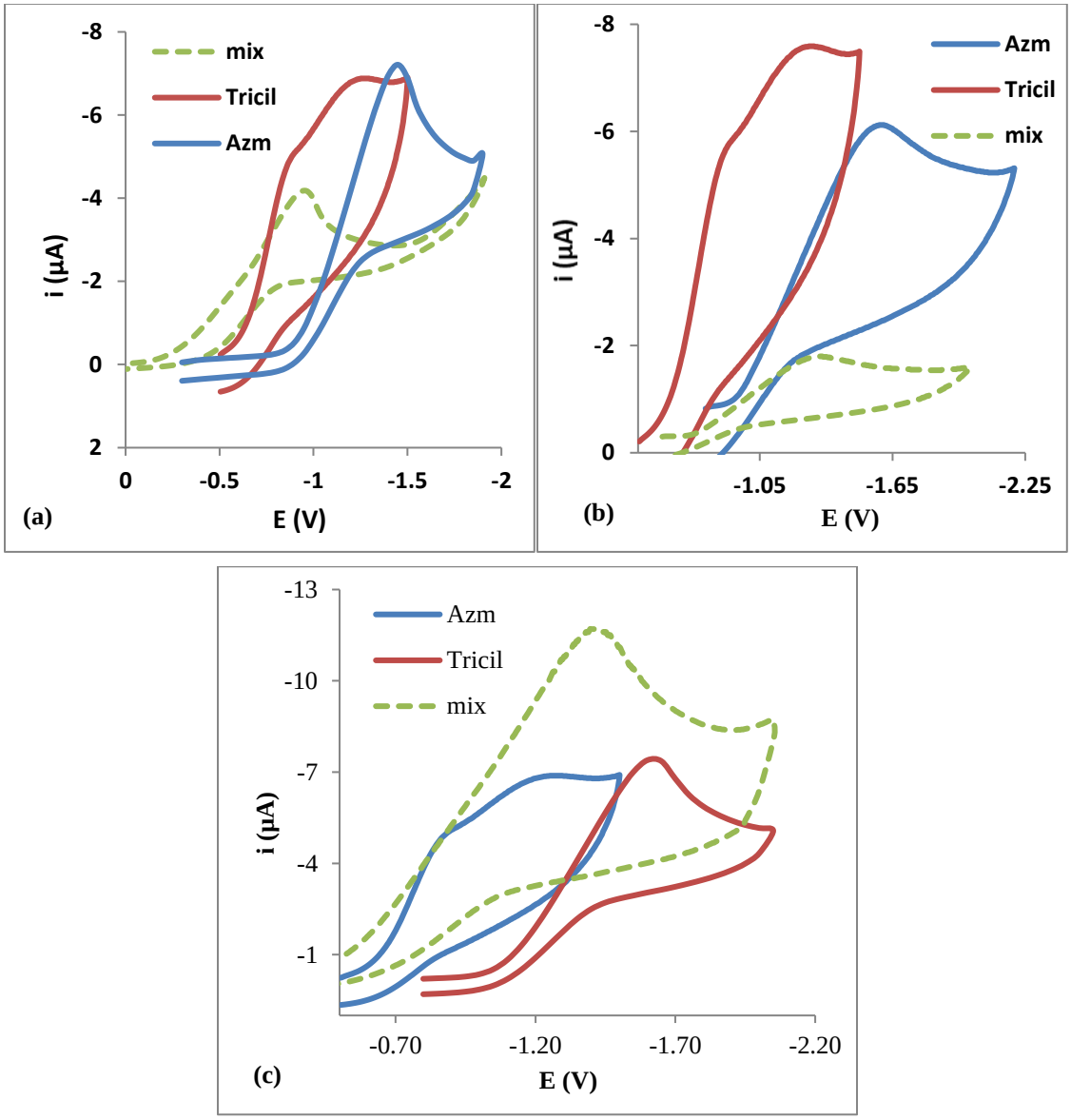


Fig. 4 Cyclic voltammograms of Azm-tricil mixture at (a) pH = 3.0. (b) pH = 9.5 and (c) pH = 7.0.

A shift to more positive potentials with a decrease of the current was observed at pH = 3 upon addition of Azm to tricil in the potential range of 0 to -2 V (Fig. 5).

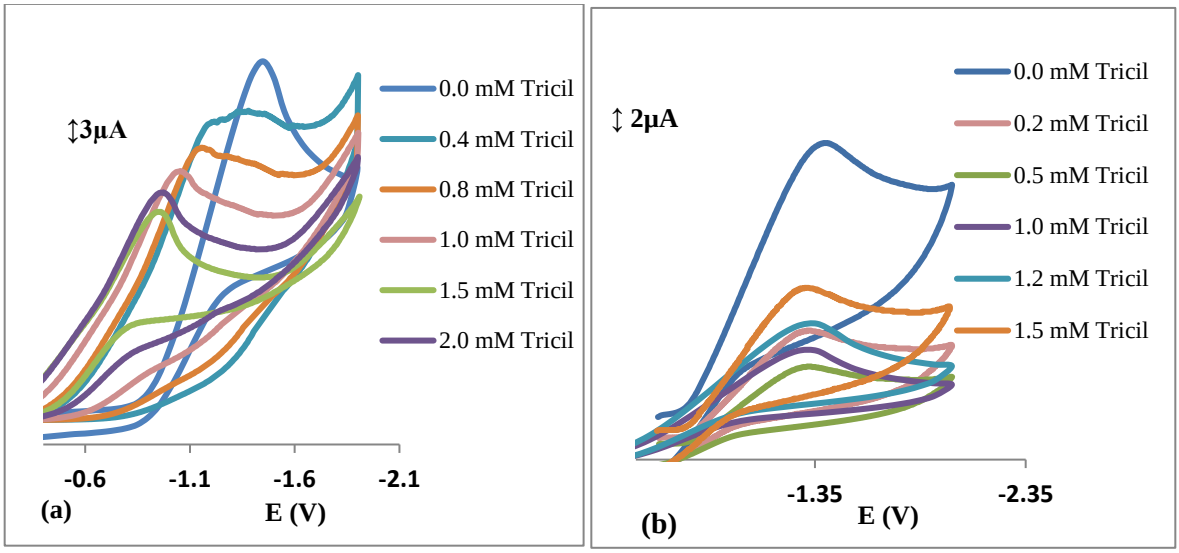


Fig. 5 Cyclic voltammograms of Azm titration with tricil at (a) pH = 3.0 and (b) pH = 9.5.

At pH = 9.5 there was a significant decay in peak current with increasing the concentration of either component. With incremental addition of tricil to 1mM Azm there was a decrease in the peak current up to certain limit after which the current increases (Fig.5 (a)) whereas with the incremental addition of Azm in 1mM Tricil the current decreased with increasing tricil concentration (Fig.5 (b)).

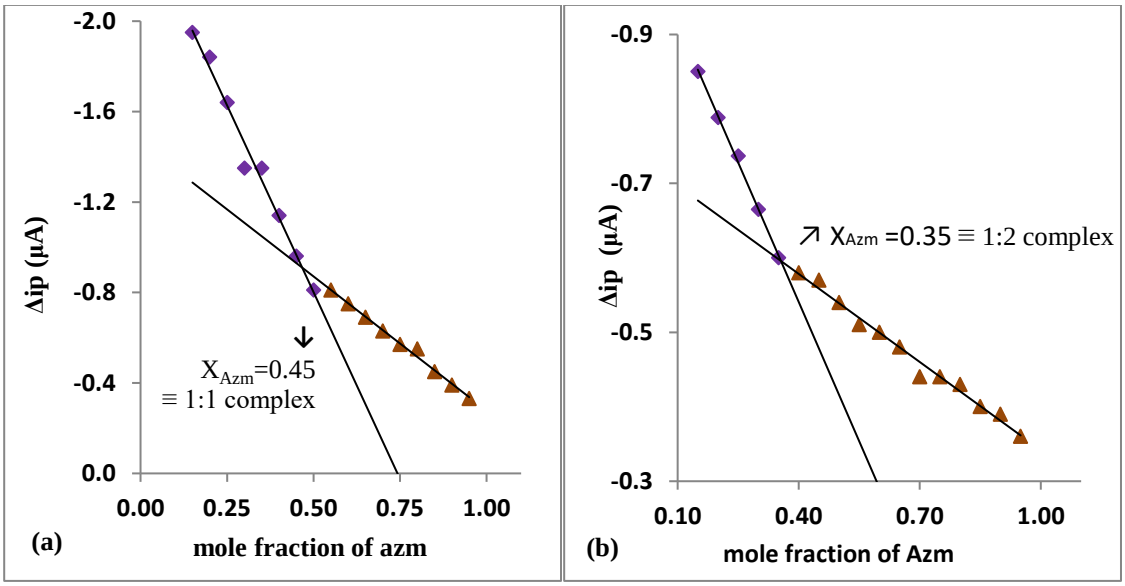


Fig. 6 Job type plots for mixture of Azm-tricil mixture at (a) pH = 3.0 (b) pH = 9.5

This decrease in current with tricil concentration was more pronounced below tricil to Azm ratio of 2. When increase in current was plotted as a function of Azm mole fraction at either pH two intersecting lines with different slopes were obtained with the intersection point in good agreement with composition of complex predicted by Jobs method in spectroscopic measurement (Fig. 6).

When the peak current was plotted as function of square root of scan rate, a straight line was obtained (data not shown here) in accordance with:

$$I_p = 2.876 \times 10^5\, n\, (\alpha n_a)^{\frac{1}{2}} A\, C_o\, D0^{\frac{1}{2}}\, \nu^{\frac{1}{2}} \qquad (2)$$

The slope of this straight line was then used to calculate diffusion constant.

$$slope\ of\ Ip\ vs\ \nu^{\frac{1}{2}}\ plot = 2.876 \times 10^5\, n\, (\alpha n_a)^{\frac{1}{2}} A\, C_o\, D0^{\frac{1}{2}} \qquad (3)$$

Table 2

Diffusion coefficients and heterogeneous electron transfer rate constants of analyte at 310±1 K.

Drugs	pH = 3.0		pH = 7.0		pH = 9. 5	
	D0×10^6 (cm^{2}s^{-1})	k°_{sh}×10^4 (cms^{-1})	D0×10^6 (cm^{2}s^{-1})	k°_{sh}×10^4 (cms^{-1})	D0×10^6 (cm^{2}s^{-1})	k°_{sh}×10^4 (cms^{-1})
Azm	0.514	2.552	2.061	3.331	0.136	2.037
Tricil	1.168	8.638	0.129	9.297	0.534	5.236
Azm-Tricil	0.021	0.966	1.073	1.023	0.032	0.809

Diffusion coefficient calculated for mixtures at pH = 3.0 and pH = 9.5 were low as compare to those of pure drugs at these pHs showing that the adduct is more bulky than pure drugs. Further, reduction of diffusion coefficient is higher at pH = 9.5 than that at pH = 3.0 showing the formation of heavier adduct at pH = 9.5 than at pH = 3.0. Rate constants are lower for mixture showing the reduction in kinetic feasibility of redox process by the adduct formation at pH = 3.0 and pH = 9.5. Whereas the D0 and k°_{sh} for the mixture were almost at the average of the respective values for pure Azm and pure Tricil at pH = 7.0 (Table 2).

Table 3

Formation constants and ΔG obtained from both techniques at pH = 3.0 and 310K ±1K.

Drug adducts	UV-Vis spectroscopy		Cyclic voltammetry	
	K_f (M^{-1})	ΔG (kJ/mol)	K_f (M^{-1})	ΔG (kJ/mol)
Azm-Tricil (pH = 3.0)	666	-16.76	625	-16.59
Azm-Tricil (pH = 7.0)	0.410	2.297	0.436	-2.139
Azm-Tricil (pH = 9.5)	1650	-19.09	1590	-18.99

The formation constants obtained from cyclic voltammetry were higher at pH = 9.5 as compared to the formation constants at pH = 3.0 as previously obtained through UV-Vis spectroscopy. ΔG was positive at pH = 3.0

and pH = 9.5 while ΔG was negative at pH = 7.0. The formation constants and free energy change for drug-drug complex formation obtained from both techniques were in agreement with each other (Table 3).

Conclusion

The Azm interacted with tricil at pH = 3.0 and pH = 9.5 but not at pH = 7.0. This revealed that certain ion formations may be involved in adduct formation that is why adduct is not being formed in neutral pH condition (pH =7.0).The strength of interaction was calculated in terms of adduct formation constant the values of which were calculated through UV-Vis spectroscopy and cyclic voltammetry. The results obtained from both techniques are in agreement with each other. Azm-tricil adduct was formed in 1:1 stoichiometry at pH = 3.0 while it exhibited 1:2 stoichiometry at pH = 9.5 as obtained from both UV-Vis spectroscopy and cyclic voltammetry.

Author(s) Contribution Statement Both the authors contributed equally to this work.

Conflict of Interest The authors declare that they have no conflict of interest.

Acknowledgements The authors highly acknowledge Department of Chemistry, Quaid-i-Azam University, Islamabad.

References

Bhattacharya, S., Banerjee, M., & Mukherjee, A. K. (2001). Study of the formation equilibria of electron donor–acceptor complexes between [60] fullerene and methylbenzenes by absorption spectrometric method. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *57*(7), 1463-1470.

Blachier, F., Davila, A. M., Mimoun, S., Benetti, P. H., Atanasiu, C., Andriamihaja, M., ... & Tomé, D. (2010). Luminal sulfide and large intestine mucosa: friend or foe?. Amino acids, 39(2), 335-347.

Fereidoonnezhad, M., Niazi, M., Shahmohammadi Beni, M., Mohammadi, S., Faghih, Z., Faghih, Z., & Shahsavari, H. R. (2017). Synthesis, Biological Evaluation, and Molecular Docking Studies on the DNA Binding Interactions of Platinum (II) Rollover Complexes Containing Phosphorus Donor Ligands. *Chem Med Chem*, *12*(6), 456-465.

Gandhi, N. R., Nunn, P., Dheda, K., Schaaf, H. S., Zignol, M., Van Soolingen, D., & Bayona, J. (2010). Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *The Lancet*, *375*(9728), 1830-1843.

Grasela, T. H., Schentag, J. J., Sedman, A. J., Wilton, J. H., Thomas, D. J., Schultz, R. W., ... & Kinkel, A. W. (1989). Inhibition of enoxacin absorption by antacids or ranitidine. *Antimicrobial agents and chemotherapy*, *33*(5), 615-617.

Henriksson, K., From, J., & Stratelis, G. (2014). Patient-reported adherence to coprescribed proton pump inhibitor gastroprotection in osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis patients using nonsteroidal anti-inflammatory drugs. Patient preference and adherence, 8, 1611.

Hussain, F. I. D. A., Arayne, M. S., & Sultana, N. A. J. M. A. (2006). Interactions between sparfloxacin and antacids--dissolution and adsorption studies. Pakistan journal of pharmaceutical sciences, 19(1), 16-21.

Jabeen, E., Qureshi, R., & Shah, A. (2013). Interaction of antihypertensive acetazolamide with nonsteroidal anti-inflammatory drugs. *Journal of Photochemistry and Photobiology B: Biology*, *125*, 155-163.

Kalanur, S. S., Katrahalli, U., & Seetharamappa, J. (2009). Electrochemical studies and spectroscopic investigations on the interaction of an anticancer drug with DNA and their analytical applications. Journal of Electroanalytical Chemistry, 636(1-2), 93-100.

Kreuter, M., Wuyts, W., Renzoni, E., Koschel, D., Maher, T. M., Kolb, M., ... & Costabel, U. (2016). Antacid therapy and disease outcomes in idiopathic pulmonary fibrosis: a pooled analysis. *The Lancet Respiratory Medicine*, *4*(5), 381-389.

Li, S. Y., Guo, Q. L., Yuan, W., Hou, Y. C., & Du, L. M. (2010). Spectrophotometric study of the charge transfer complexation of some amino acid derivative drugs as electron donors with 7, 7, 8, 8-tetracyanoquinodimethane. *Bulletin of the Chemical Society of Ethiopia*, *24*(1).

Marieb, E. N., & Hoehn, K. (2010). The integumentary system. Human Anatomy and Physiology. 8th ed. San Francisco, CA: Benjamin Cummings, 155.

Marques, M. R., Loebenberg, R., & Almukainzi, M. (2011). Simulated biological fluids with possible application in dissolution testing. Dissolution Technol, 18(3), 15-28.

Mihaly, G. W., Marino, A. T., Webster, L. K., Jones, D. B., Louis, W. J., & Smallwood, R. A. (1982). High dose of antacid (Mylanta II) reduces bioavailability of ranitidine. *Br Med J (Clin Res Ed)*, *285*(6347), 998-999.

Parameshappa, B., Rao, N. V., Gouda, T. S., Sen, S., Chakraborty, R., Basha, M. A., ... & Kumar, S. S. (2010). A study on drug-drug interaction between anti-hypertensive drug (propranolol) and anti-diabetic drug (glipizide). Annals of Biological Research, 1(3), 35-40.

Sadowski, D. C. (1994). Drug interactions with antacids. *Drug Safety*, *11*(6), 395-407.

Srinivasan, V. S. (2001). Bioavailability of nutrients: a practical approach to in vitro demonstration of the availability of nutrients in multivitamin-mineral combination products. *The Journal of nutrition*, *131*(4), 1349S-1350S.

Teleb, S. M., & Refat, M. S. (2004). Spectroscopic studies on charge-transfer complexes formed in the reaction of ferric (III) acetylacetonate with σ-and π-acceptors. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 60(7), 1579-1586.

Uhde, M., Ajamian, M., Caio, G., De Giorgio, R., Indart, A., Green, P. H., ... & Alaedini, A. (2016). Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. Gut, gutjnl-2016.