Synthesis, characterization and enzyme inhibitory activity of new pyrazinamide iron complexes

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Abstract: The present paper deals with synthesis, characterization and amylase inhibitory activity of pyrazinamide (PYZ) with iron in its both (II) and (III) oxidation states. The synthesized complexes were characterized on the basis of IR, UV, ^{1}H-NMR, ^{13}C-NMR, elemental analysis and SEM. Changes in IR data shows that PYZ form complex with octahedral geometry and binding sites are ring nitrogen and carbonyl group, wherein two sides are satisfied with two chloride ions. SEM images indicate the crystalline state and surface morphology of PYZ and its complexes. Elemental analysis proves the composition of complexes. Pyrazinamide and the complexes showed no significant effect on amylase activity but the activity was inhibited in the presence of ferrous chloride.

Keywords: Pyrazinamide, amylase, ferrous chloride, ferric chloride, oxidation state.

INTRODUCTION

Albeit tuberculosis has been declared by WHO to be a global emergency (Lavor *et al.,* 2014), pyrazinamide (PYZ, fig. 1) is being used as a as first line drug to treat tuberculosis from almost 50 years (Akyuz, 2003; Castro *et al.,* 2009; Singh *et al.,* 2006; Lemaitre *et al.,* 2001).

PYZ acts as bactericidal for mycobacteria and reduce treatment time up to six month from 9-12 month (Jurca and Marian 2009; Zhang *et al.,* 2003) because it kills a population of semi-dormant tubercle bacilli in acidic pH environments that are not killed by other tubercular drugs. Pyrazinamide is a paradoxical and unconventional drug (Zhang *et al.,* 2003).

Pyz is used along with Isoniazid, Ethambutol and Rifampicin (Akyuz, 2003; Zhang *et al.,* 2003; Somoskovi *et al.,* 2004; Gunasckaran and Sailatha 2009) in different dosage forms and is available in market with different brand name. It is an analog of nicotinamide, has a carbonyl, amino group with two electron donating ring nitrogen which behave as a ligand and for complexes with transition metals.

It is evident that the parent molecule and some of its complexes are widely used due to their anti-bacterial properties (Akyuz, 2003) and play an important role in biological activity of drugs (Tiwari, 2012; Magare *et al.,* 2009) as well as effect on the activity of enzymes.

Human amylase acts on α 1,4-β glycosidic linkage in the polysaccharide (Frossard *et al.,* 2008) and plays an important role in providing cells with glucose, energy and carbon source (Meisler and Ting 1993). Amylase is basically a metallo-enzyme that requires calcium to perform enzyme activity. The calcium increase enzyme stability and prevent proteolysis of amylase (Hsiu *et al.,* 1964). The influence of different metals on the activity of amylase varies from one another (Li *et al.,* 2007). Previously, it was reported that a number of metals including silver, mercury, cadmium, copper, lead, iron, nickel, manganese and zinc inhibited amylase activity (Pandey *et al.,* 2000; Gupta *et al.,* 2003; Sun *et al.,* 2010). However, some others are reported to be the strong activators that include calcium, cobalt and barium (Metin *et al.,* 2010). The current study is designed to observe the effect of the pyrazinamide, ferrous and ferric ions; and their complex on the activity of amylase enzyme.

MATERIALS AND METHODS

Reagents

Pure samples of pyrazinamide was gifted by Wyeth Pakistan Ltd. and used as ligands. Ferric chloride $FeCl_3.6H_2O$, Ferrous chloride $FeCl_2.4H_2O$, methanol, ethanol, isopropyl alcohol, butanol, dimethyl sulfoxide (DMSO), dimethyl formaide (DMF), ethyl acetate and chloroform were used. All reagents used were of A.R Grade.

Instrumentation

Elemental analyses (CHN) were carried out on a Perkin Elmer 2400 Series II. The IR spectra were recorded in KBr pellets with a Shimadzu Prestige -21 spectrophotometer in the 4000-400cm^{-1} range. The UV visible spectra were recorded on a Shimadzu UV-1601 spectrophotometer in CH_3OH. Metals were estimated by using standard methods (Vogel, 1959; Kohlhoff and Stegar, 1947; Coratt, 1955). SEM images were taken by Scanning Electron Microscope, JEOL JAPAN model no.

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JSM6380A with auto-coater JEOL JAPAN model no. JFC1500, Magnetic susceptibility values were measured at room temperature with Mark 1 Magnetic Susceptibility Balance from Sherwood Scientific. Pascal's constants were used to apply diamagnetic corrections for complexes. Conductance and pH of 0.5% solution of complexes were measured in DMSO by conductivity meter Janway 4071 and METLLER Toledo MP220 pH meter.

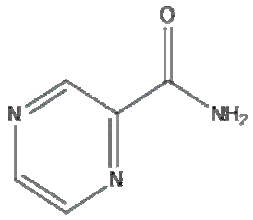

Fig. 1: Structure of pyrazinamide

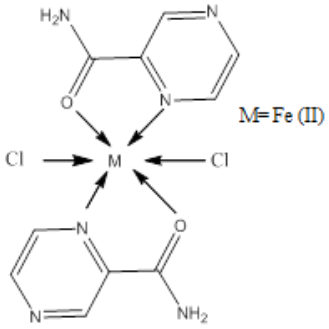

Fig. 2: Structure of Fe (II) pyrazinamide complex

Synthesis of the complexes

A warm methanolic solution of 0.1 M pyrazinamide (0.1 M; 20 mL) and metal salts of $FeCl_2$ and $FeCl_3$ (0.1 M; 10 mL) solutions were mixed in separate round bottomed flasks and refluxed for three hours on a water bath with time to time stirring. Finally, solid complexes were formed which were filtered and washed with hot solvent, worked up and dried at 60°C. It was observed that colored complexes were formed as compared to the ligand. The colors of the formed complexes are listed in table 1.

Effect of pyrazinamide and its complexes on the activity of amylase enzyme

100 µ/mg of amylase enzyme mixed with 1mM of pyrazinamide and its complexes separately. The mixture was incubated at 37°C for 2 hours after which enzyme activity of amylase was determined by the method (Maria *et al.,* 2013). One unit of enzyme was defined as amount of enzyme required to release 1.0µM of glucose by hydrolyzing starch under standard condition.

RESULTS

Values of Magnetic susceptibility, conductance and pH of complexes are mentioned in table 1. The complexes of pyrazinamide have been synthesized with 1:2 mole ratios (fig. 2-3) All complexes are stable at room temperature. Solubility of the complexes was found in different solvent, solubility, colors and melting points of formed complexes as compared to ligands are listed in table 1. The structure of the complexes established from the elemental analysis agrees well with their proposed formulas in table 2.

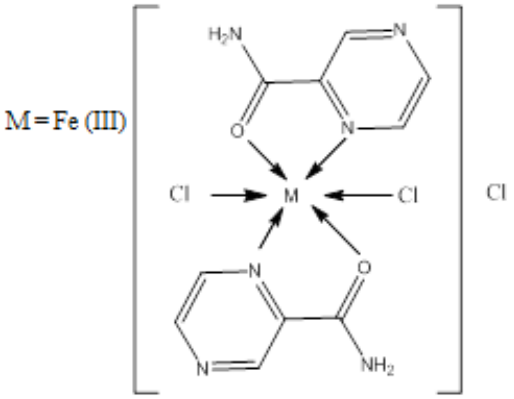

Fig. 3: Structure of Fe (III) pyrazinamide complex

FTIR spectra

The most characteristic peaks of IR spectra of pyrazinamide and its complexes are shown in table 3. Pyrazinamide can bind the metal from three sides, C=O, Ring Nitrogen and from Amide NH_2, N-H symmetric stretching band in pyrazinamide appears at 3289 (Gunasckaran and Sailatha, 2009), 3140 (Jurca and Marian, 2009), 3290 (Akyuz, 2003) but in current study band appear at 3140cm-^{1}, N-H asymmetric stretching band in pyrazinamide appear at 3414 (Gunasckaran and Sailatha, 2009), 3410 (Jurca and Marian, 2009), 3412 (Akyuz, 2003) in this study band appear at 3437cm-^{1}, C=O band in pyrazinamide appear at 1714 (Gunasckaran and Sailatha, 2009), 1705 (Jurca and Marian, 2009), 1714 (Akyuz, 2003) and in this experiment band appear at 1686 cm-^{1}, ring C=N symmetric stretching band in pyrazinamide appear at 1378 (Gunasckaran and Sailatha, 2009), 1375 (Jurca and Marian, 2009), 1378 (Akyuz, 2003) but in this study band appear at 1379cm-^{1}, ring C=N asymmetric stretching band in pyrazinamide appear at 1437 (Gunasckaran and Sailatha, 2009), 1437 (Akyuz, 2003) and in this study band appear at 1437cm-^{1}, ring C-C asymmetric stretching band in pyrazinamide appear at 1524 (Gunasckaran and Sailatha, 2009), 1570 (Jurca and Marian, 2009), 1589 (Akyuz, 2003) but in this study band appear at 1604 cm-^{1} (fig. 4 and 5).

Earlier studies shows synthesized pyrazinamide complexes of transition metal (II) tetracyanonickelate and proposed PYZ coordinate metal from carbonyl carbon (Akyuz, 2003). Tünde Jurca *et al.,* suggested that the binding side in PYZ are ring nitrogen and carbonyl group and form five member ring but in this study, IR spectra

Table 1: Basic physical characteristics of pyrazinamide iron complexes

Compound	Color	M.P (^{O}C)	B.M	Conductance (ms) 0.5%	pH 0.5%	Solubility
Pyrazinamide (Pyz)	White	190	-	-		H_2O, CH_3OH, DMF and DMSO
$[Fe(II)(PYZ)_2(Cl)_2]$	Reddish Brown	202 D	4.81	0.74	2.46	H_2O and CH_3OH
$[Fe(III)(PYZ)_2(Cl)_2]Cl$	Reddish Brown	255 D	5.14	0.95	2.06	H_2O and CH_3OH

Table 2: Elemental analysis of pyrazinamide iron complexes

Compound	% C		% H		% N	
	Theoretical	Practical	Theoretical	Practical	Theoretical	Practical
Pyrazinamide Pyz	48.78	48.66	4.06	3.98	34.15	34.26
$[Fe(II)(PYZ)_2(Cl)_2]$	32.17	32.29	2.68	2.59	22.52	23.47
$[Fe(III)(PYZ)_2(Cl)_2]Cl$	29.39	29.79	2.45	2.04	20.57	20.98

Table 3: Infrared spectral assignments of pyrazinamide iron complexes

Peak Assignment	C=O	N-H Asymmetric Stretching	N-H Symmetric Stretching	Ring C-C Symmetric stretching	Ring C-C Asymmetric stretching	Ring C=N Symmetric stretching	Ring C=N Asymmetric stretching
Pyrazinamide	1686	3437	3140	1506	1604	1379	1437
$[Fe(II)(PYZ)_2Cl_2]$	1709	3439	3145	1508	-	1381	1439
$[Fe(III)(PYZ)_2Cl_2]$	1711	3441	3142	1510	-	1381	1435

show C=O appear at 1705cm-^{1} in PYZ and at 1700cm-^{1} in Cu-PYZ complex (Jurca and Marian, 2009) which show no blue shift in C=O peak and no support the proposed structure whereas in this study $\Delta\nu$ in C=O peaks of ligand and complexes 25 cm-^{1} which is suggests that the binding side of complex is from oxygen of C=O group of PYZ.

UV Visible spectra

UV Visible spectra of pyrazinamide and its complexes were observed in CH_3OH (fig. 6). PYZ shows three λ_{max} at 208, 269 and 319 nm due to π to π^* transition of C=N and C=O. $FeCl_2$ shows two λ_{max} at 365 and 246 nm due to ^{1}A1g to ^{1}T1g and $^{1}A_{1g}$ to $^{1}T_{2g}$. $FeCl_3$ also shows three λ_{max} at 362, 250 and 216 nm due to $^{2}T_{2g}$ to $^{2}A_{2g}$, $^{2}T_{1g}$, $^{2}T_{2g}$ to $^{2}E_g$ and $^{2}T_{2g}$ to $^{2}A_{1g}$.

SEM images

Scanning Electron Microscope (SEM) produced images with a focused beam of electrons evident of the crystal morphology of compounds. SEM technique gives a general perception about microstructure, surface morphology, particle size, and chemical composition (Al-Saif and Refat, 2013) of respective free PYZ ligand, $[Fe(II)(PYZ)_2Cl_2]$ and $[Fe(III)(PYZ)_2Cl_2]Cl$ complexes. fig. 7 designed the SEM images of the free ligand and synthesized PYZ complexes at various magnification levels.

Influence of pyrazinamide and complexes on amylase activity

When pyrazinamide was incubated with amylase it was observed that there was no significant effect on the activity of amylase and remained unchanged. However, in the presence of ferrous chloride ($FeCl_2$) amylase activity was decreased and the same result was observed when amylase was incubated with pyrazinamide complex of ferrous (fig. 8a). Similar inhibitory results of ferrous against amylase were reported (Hsiu *et al.,* 1964; Sindhu *et al.,* 2011; Singh *et al.,* 2015). In case of ferric ions similar inhibitory pattern was observed by ferric chloride as well as with the pyrazinamide complex with ferric chloride (fig. 8b).

DISCUSSION

In Fe (II)-PYZ complex, PYZ behave as a weak field ligand so only one transition is observed at 500.7 nm due to 5T2g to 5Eg and in Fe (III)-PYZ also one peak observed at 497.8 nm this may be due to 2T2g to 2A2g, 2T1g. The band shifting in both complexes shows the formation of complexes (fig. 6). The uniformity and similarity between the particles forms of synthesized PYZ complexes indicate that the existence of morphological phases of [Fe(II)(PYZ)2Cl2] and [Fe(III)(PYZ)2Cl2]Cl complexes have a homogeneous matrix and have more crystalline as compare to mother compound (fig. 7).

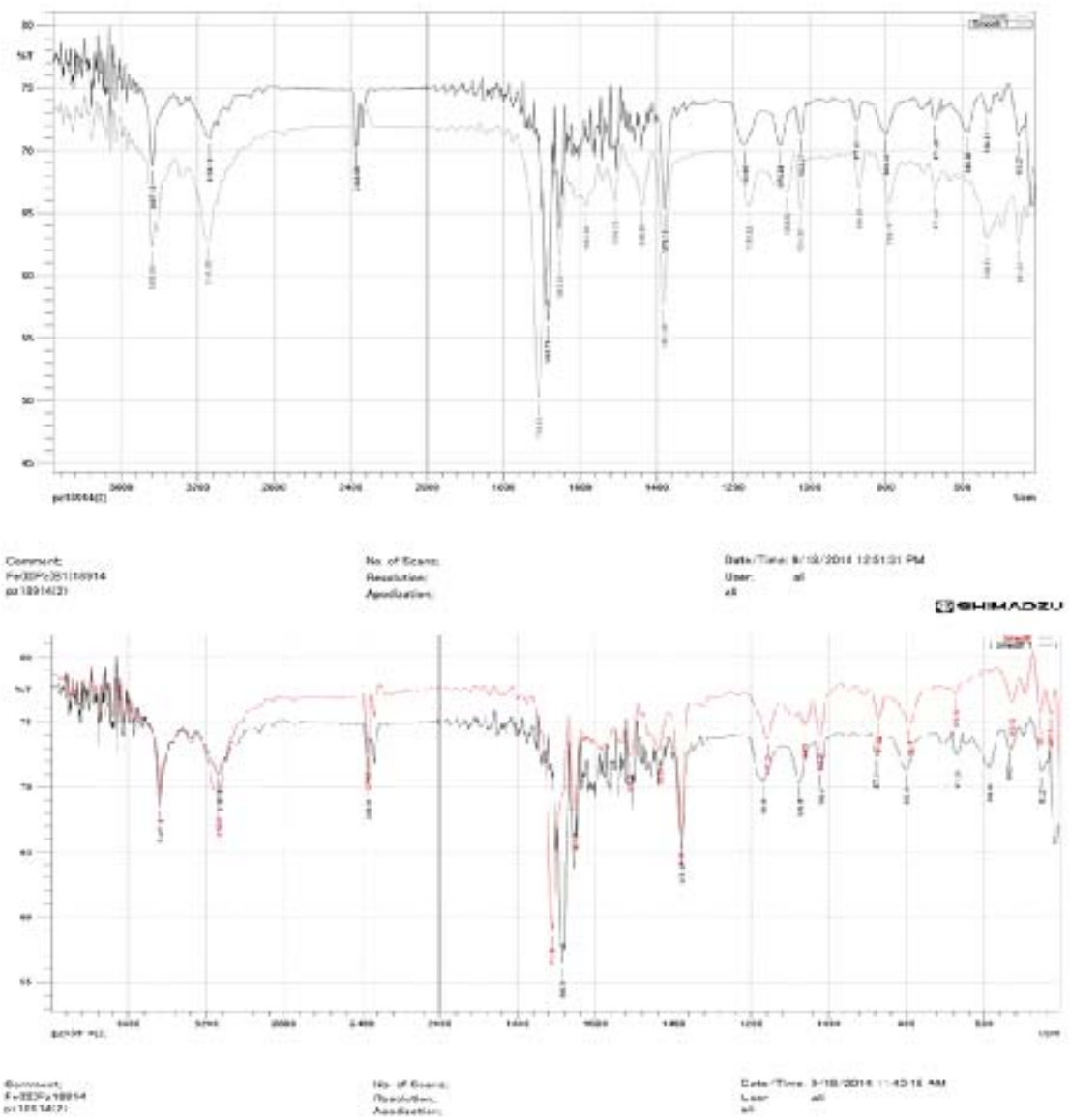

Fig. 5: FTIR spectra of Fe(III)PZ complex (Red) and PZ (black)

The results indicated inhibitory effect of iron on amylase activity. It was reported that amylases are classified as metallo enzymes due the presence of metal in the molecule that contribute during enzymatic reaction. In the presence of iron competition may exist among the two metallic ions that reduced amylase activity (Sindhu *et al*., 2011).

CONCLUSION

Pyrazinamide is an important anti tuberculosis drug and its complexes were synthesized using iron in two different oxidation state. The complexes were characterized by different techniques. When pyrazinamide and its complexes were incubated with amylase it was observed that there was no significant effect on the activity of amylase and remained unchanged.

This indicate that Pz itself have no inhibitory effect on amylase activity but when the complexes are synthesized with iron in two different oxidation state, the enzyme inhibition was observed. It was due to the fact that iron in both state (I, II) inhibit the activity of amylase and the reason might be that in the presence of iron competition may exist among the two metallic ions that reduced amylase activity. Therefore, it can concluded that these complexes can be used for the treatment but further research is needed to explore other efficacy of the synthesized complexes.

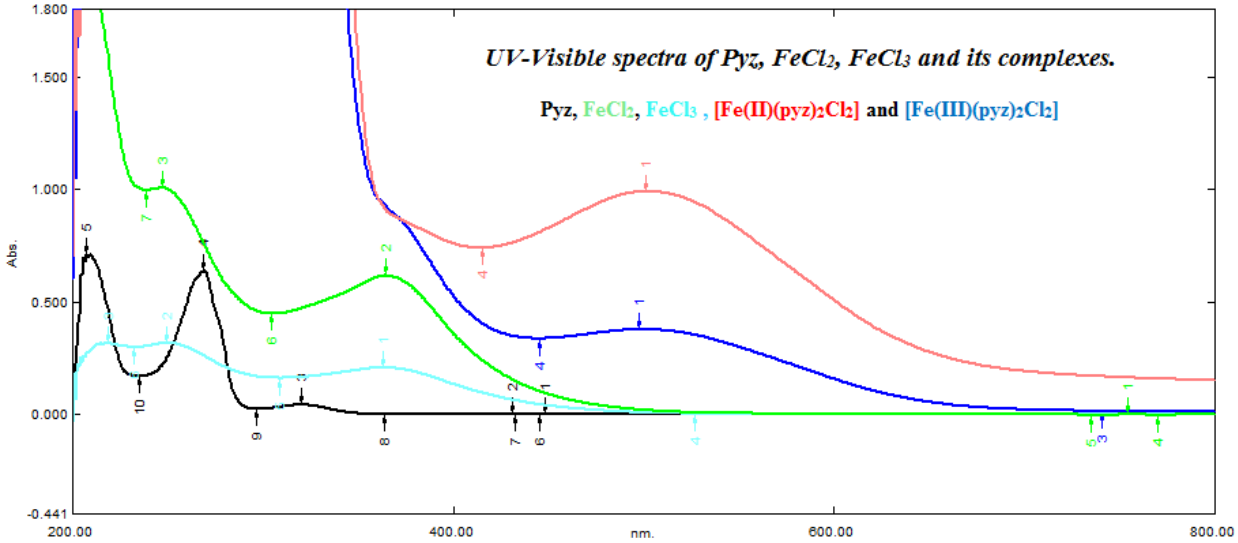

Fig. 6: UV- visible spectra of Pyz, FeCl2, FeCl3, and its complexes.

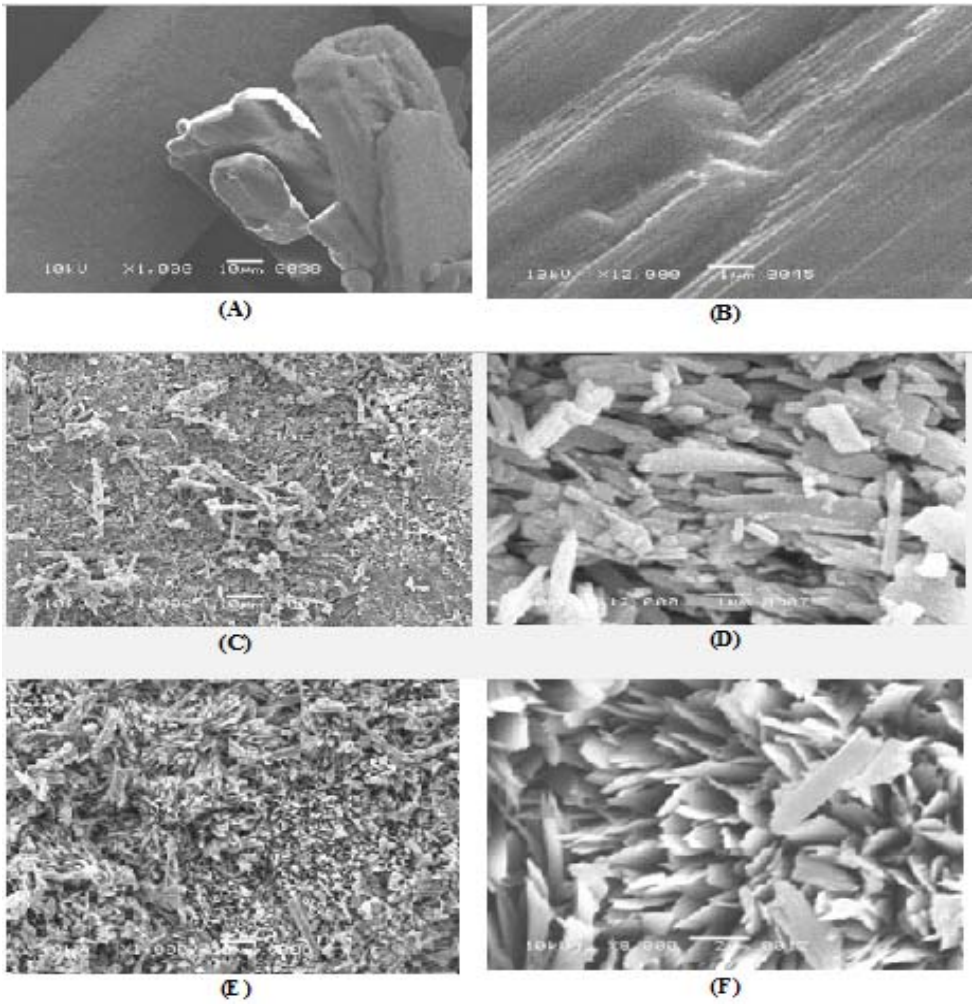

Fig. 7: SEM images of pyrazinamide and its complexes. (A) SEM Image PYZ at 1,000 magnification. (B) SEM Image PYZ at 12,000 magnification. (C) SEM Image $[Fe(II)(PYZ)_2Cl_2]$ at 1,000 magnification. (D) SEM Image $[Fe(II)(PYZ)_2Cl_2]$ at 12,000 magnification. (E) SEM Image $[Fe(III)(PYZ)_2Cl_2]$ at 1,000 magnification. (D) SEM Image $[Fe(III)(PYZ)_2Cl_2]$ at 12,000 magnification.

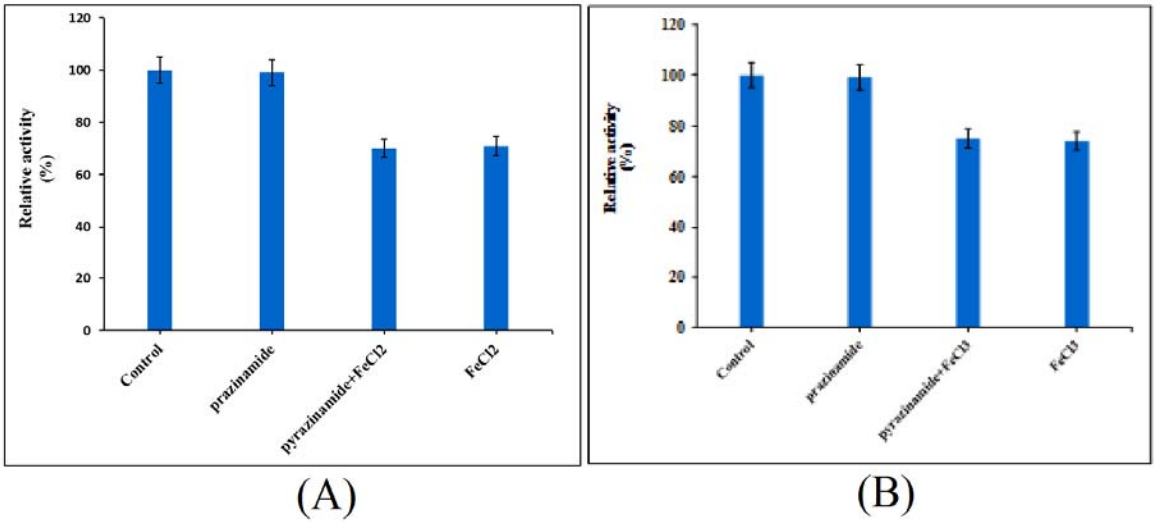

Fig. 8: Effect of amylase on drug and their derivatives (A) effect of pyrazinamide and its complex with ferrous chloride (B) effect of pyrazinamide and its complex with ferric chloride.

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