

Scientific paper

pH Metric, Spectroscopic and Thermodynamic Study of Complexation Behavior of 2-aminobenzothiazole with Ni (II) in Presence of Amino Acids

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Abstract

The complexation of 2-aminobenzothiazole (2abt) [A] with Ni(II) in presence of amino acids viz., glycine (gly), L-alanine (ala), L-valine (val) and L-phenylalanine (pal) [B] in 50% (v/v) water-ethanol mixture containing NaClO₄ (0.15 M) has been studied by pH metrically at various temperatures (300, 310, 320 and 330 ± 0.1 K). Mixed ligand complexes of types NiAB and NiAB₂ were observed and their stability constants were determined. The stabilization of mixed ligand complexes over binary analogues has been derived from $\Delta \log K$, $\log X'$ and $\log X$ values. The thermodynamic parameters ΔG , ΔH and ΔS were derived from the temperature dependence of the equilibrium constants. The complexation behavior has also been studied by means of electronic spectra. On the basis of stability constants and electronic spectra, it is revealed that the mixed ligand complexes have six-coordinated octahedral structure. The binary and mixed ligand complexes were screened for their microbial activities *in vitro* on common bacteria, fungi and yeast. The DNA cleaving activities were studied by electrophoresis method.

Keywords: Mixed ligand complexes, 2-aminobenzothiazole, amino acids, stability constants and biological screening.

1. Introduction

In recent years, the importance of chemical equilibrium modeling has been developed from an empirical qualitative tool to a sophisticated quantitative tool for chemists¹. Thiazoles are a crucial part of vitamin B1 and found in cocarboxylase coenzyme². Benzothiazoles are used in pharmacology and cancer biology³. Mixed ligand complexes of amino acids are involved in the exchange and transport mechanism of trace metal ions in the human body⁴. Much attention has been paid to the study of mixed ligand complexes of transition metals with ligands of biological and pharmaceutical interest^{5,6}. In a sequel of continuation, the major goal of the present work is to determine stability constants of various species present in the Ni(II)-2abt (A)-amino acid (B) mixed ligand complexes containing 0.15 M NaClO₄ by pH-metrically at different

temperatures (300, 310, 320 and 330 ± 0.1 K). The corresponding thermodynamic functions of complexation were evaluated and are discussed. The coordination environment of Ni(II) ion in mixed ligand complexes was determined by electronic spectral measurements at different pH in 50% (v/v) water-ethanol medium. Biological activities of binary and mixed ligand complexes were tested with different microorganisms. DNA cleavage activities of binary and mixed ligand complexes were monitored by gel electrophoresis.

2. Experimental

2.1. Materials

All the chemicals used in this study were analytical grade and were used without further purification. Carbonate free sodium hydroxide solution was prepared and

standardized against standard potassium hydrogen phthalate solution. The ionic strength of each solution was adjusted to 0.15 M by the addition of NaClO₄ as supporting electrolyte. The metal perchlorate and other reagents were prepared and estimated as described elsewhere.^{7,8}

2. 2. Potentiometric Equilibrium Measurements

The pH titrations were carried out in a digital pH meter (Systronics µpH System 361) with a combined glass electrode (accuracy ± 0.01 pH unit). The temperature of the sample solutions was maintained at 300, 310, 320 and 330 ± 0.1 K. In both the acidic and alkaline regions, the electrode system was calibrated in terms of hydrogen ion concentrations instead of activities. The instrument was calibrated using standard buffer solutions⁹. The stability constants were evaluated with the aid of SCOGS computer program¹⁰. In this study the selection of 'Best Fit Model' is based on the factors such as (i) the analysis of formation curves; (ii) standard deviation in titre with 0.02 cc which is within 0.05% error with respect to total volume of the titre and compares well with σ_v obtained for ligand protonation constants under similar conditions and (iii) minimum standard deviation in the log β values of the individual species. In addition to these factors the chemical logic as also been taken into consideration in the selection of the 'Best Fit Model'. Oxygen free nitrogen gas was bubbled through the solution before and during titrations. Multiple titrations were carried out for each system. The pH-meter readings in 50% (v/v) water-ethanol mixture were corrected by Van Uitert and Hass relation¹¹. The ion product of water ($K_w = [H^+][OH^-]$) were calculated at constant ionic strength of 0.15 mol L⁻¹ with NaClO₄ in 50% (v/v) water-ethanol mixture based on the measurement of $[H^+]$ and $[OH^-]$ and pH in several experiments. The K_w obtained is 14.42 (±0.03) at 27 °C and in agreement with the literature value^{12,13}. Spectrophotometric determinations in the visible region were performed with Jasco 430 UV-visible spectrophotometer in the range of 200–1100 nm at 310 K.

2. 3. Biological Studies

The *in vitro* biological activity of binary and mixed ligand complexes were tested against the bacteria, fungi and yeast by a modified well diffusion method¹⁴. Commercially available ampicillin and nystatin were used as antibacterial and antifungal control respectively. The test solutions of Ni(II)-2abt-gly/ala/val/pal (1:1:1) [3×10^{-3} M] were prepared by dissolving the mixed ligand complexes in 50% (v/v) water-ethanol mixture. The bacteria were cultured for 24 h at 37 °C in an incubator. The compounds to be tested were added to a 10 mm diameter well and the plates were then kept at 37 °C in an incubator. The growth

of inhibition zones was measured and is compared with control.

2. 4. DNA Cleavage Studies

The DNA cleavage activity of binary and mixed ligand complexes was monitored by agarose gel electrophoresis on CT DNA. The gel electrophoresis experiments were performed under aerobic conditions with H₂O₂ as oxidant by incubation at 37 °C for 2 h as follows: CT DNA 30 µM, 50 µM of each mixed ligand complex, 500 µM of H₂O₂ in 50 mM Tris-HCl buffer (pH = 7.2). After incubation, 1 µL of loading buffer (bromophenol blue in H₂O) was added to each tube and the mixed samples were loaded on 1% agarose gel. The samples were electrophoresed for 2 h at 50 V in Tris-acetic acid – EDTA buffer (pH = 8.3). After electrophoresis, the gel was stained with 1 µg/cm³ ethidiumbromide (EB) for 30 min prior to being photographed under UV light.

3. Results and Discussion

3. 1. Stability and Structure of Binary Species

The protonation constant of 2abt and the stability constant of Ni(II)-2abt(A) binary complex were determined by pH-metrically at different temperatures using NaClO₄ as supporting electrolyte. The stability constant values are given in Table 1. The pK_a value of 3.94 for 2abt compares very well with the value of 3.92 of thiazole¹⁵. The log β_{NiA} and log β_{NiA_2} values obtained in Ni(II)-2abt are 4.09 and 6.44 respectively at 300 K. The pK_a value of amino group in 2abt is in the range of 10–11. The amino nitrogen atom coordinates with metal ion only at higher pH. In the present investigation the titration was carried out up to 8 pH, and increasing the pH above 8 leads to precipitation rules out the possibility of involvement NH₂ nitrogen in coordination with metal ion. This shows that 2abt binds the metal ion via thiazole ring nitrogen atom. This type of binding has already been established in Ni(II)-2-aminobenzothiazole derivatives in their solid state¹⁶. The stability constants of amino acids were redetermined under the present experimental conditions and the values agree well with the reported values^{17–19}. The amino acids bind Ni(II) ion in a bidentate manner through carboxylate oxygen and amino nitrogen atoms forming a 5-membered ring.

3. 2. Stability and Structure of Mixed Ligand Species

In Ni(II)-2abt(A)-amino acid(B) systems, NiAB and NiAB₂ species have been identified. The log $K_{NiAB}^{NiB}/\log K_{NiAB_2}^{NiB_2}$ values obtained at different temperatures in Ni(II)-2abt(A)-amino acid(B) systems compare favorably with

Table 1. Stability constants for the proton and parent binary complexes of Ni(II) with 2abt*(A), gly, ala, val and pal (B) in 50% (v/v) water – ethanol mixture at 300, 310, 320 and 330 K, I = 0.15 M (NaClO₄)

Temp(K)	Parameters	2abt*	gly	ala	val	pal
300	$\log \beta_{\text{HA}}$	3.94(3)	9.77(2)	10.07(2)	10.11(3)	9.55(3)
	$\log \beta_{\text{H}_2\text{A}}$	---	12.05(3)	12.38(4)	12.59(4)	11.67(5)
	$\log \beta_{\text{NiA}}$	4.09(3)	5.73(2)	5.67(3)	5.68(4)	5.52(3)
	$\log \beta_{\text{NiA}_2}$	6.44(4)	10.34(3)	10.56(4)	10.20(3)	10.01(3)
310	$\log \beta_{\text{HA}}$	3.80(3)	9.57(3)	9.72(2)	9.80(3)	9.36(4)
	$\log \beta_{\text{H}_2\text{A}}$	---	11.74(4)	12.25(3)	12.45(5)	11.50(6)
	$\log \beta_{\text{NiA}}$	4.31(4)	5.66(3)	5.55(4)	5.49(5)	5.34(4)
	$\log \beta_{\text{NiA}_2}$	6.79(5)	10.06(4)	10.27(5)	10.05(6)	9.79(6)
320	$\log \beta_{\text{HA}}$	3.67(4)	9.20(3)	9.48(2)	9.53(3)	9.19(4)
	$\log \beta_{\text{H}_2\text{A}}$	---	11.40(5)	11.92(4)	12.17(5)	11.27(5)
	$\log \beta_{\text{NiA}}$	4.56(3)	5.54(4)	5.41(3)	5.34(4)	5.25(4)
	$\log \beta_{\text{NiA}_2}$	7.10(5)	9.72(6)	10.14(5)	9.70(6)	9.47(7)
330	$\log \beta_{\text{HA}}$	3.55(3)	8.97(3)	9.30(2)	9.42(4)	8.96(4)
	$\log \beta_{\text{H}_2\text{A}}$	---	11.19(4)	11.67(3)	11.85(5)	10.82(5)
	$\log \beta_{\text{NiA}}$	4.82(4)	5.44(3)	5.31(4)	5.27(5)	5.18(5)
	$\log \beta_{\text{NiA}_2}$	7.32(5)	9.54(5)	10.02(6)	9.49(7)	9.34(6)

* 2abt becomes primary ligand (A) in the mixed ligand systems.

$\log K_{\text{NiA}}^{\text{Ni}}$ value in Ni(II)-2abt binary system (Table 1). This shows that 2abt in mixed ligand complexes binds the metal ion in a manner similar to its binding in the NiA species. Again, $\log K_{\text{NiAB}}^{\text{NiA}} / \log K_{\text{NiAB}_2}^{\text{NiA}}$ values (Table 2) obtained in NiAB / NiAB₂ systems compare favorably with $\log K_{\text{NiB}}^{\text{Ni}} / \log K_{\text{NiB}_2}^{\text{Ni}}$ values in Ni(II)-amino acid systems. This shows that the binding mode of amino acid (B) ligand in NiAB / NiAB₂ mixed ligand species is similar to its binding mode in the corresponding binary systems. Thus, the three coordinating positions in Ni(II)-2abt(A)-amino acid(B) systems would be occupied by the monodentate binding of 2abt and bidentate binding of amino acids. Solvent water molecules would occupy the remaining positions (Figure 1).

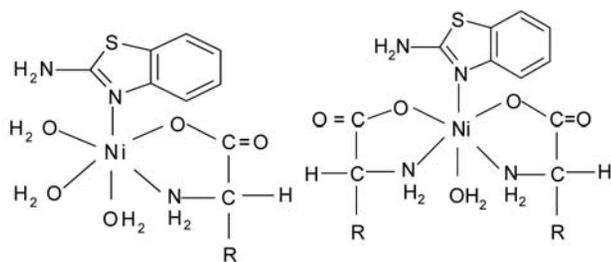


Figure 1. Proposed structures of NiAB and NiAB₂ species in Ni(II)-2abt (A) – amino acid (B) systems (R = H – glycine, CH₃ – alanine, (CH₃)₂ – valine and C₆H₅ – phenylalanine).

The stabilization of mixed ligand complexes over binary analogues can be expressed in terms of $\Delta \log K$ ($= \log \beta_{\text{NiAB}}^{\text{Ni}} - \log K_{\text{NiA}}^{\text{Ni}} - \log K_{\text{NiB}}^{\text{Ni}}$). The $\Delta \log K_{\text{NiAB}}^{\text{Ni}}$ values (Table 2) calculated for all these systems are more positive indicating the marked stabilization of mixed ligand complexes²⁰. The quantitative stabilization of mixed li-

gand complexes can be expressed by $\log X$ values²¹ ($= 2 \log \beta_{\text{NiAB}}^{\text{Ni}} - \log \beta_{\text{NiA}_2}^{\text{Ni}} - \log \beta_{\text{NiB}_2}^{\text{Ni}}$). The $\log X$ values (Table 2) are more positive compared to the statistical values²² demonstrate that interligand and electronic interactions are present in the mixed ligand complexes. The $\log X'$ ($= (\log \beta_{\text{NiAB}}^{\text{Ni}} + \log K_{\text{NiA}}^{\text{Ni}}) - (\log \beta_{\text{NiA}_2}^{\text{Ni}} + \log K_{\text{NiB}}^{\text{Ni}})$) values²³ are found to be greater than 0.3, suggesting that NiA and NiB bonds in mixed ligand complexes are stronger than in binary complexes.

3. 3. Effect of Temperature

The dissociation constants of the ligands, as well as the stability constants of the complexes with Ni(II) ions in 50% (v/v) water-ethanol mixture have been evaluated at 300, 310, 320 and 330 K (Table 1). From the results, it is clear that the $\text{p}K_{\text{HA}}$ values of the ligands decrease with increasing temperature. In Ni(II)-2abt binary system, the stability constants $\log K_{\text{NiA}}^{\text{Ni}}$ and $\log K_{\text{NiA}_2}^{\text{Ni}}$ values increase with increasing temperature. The enthalpy change (ΔH) for the dissociation and complexation process was calculated from the slope of Vant Hoff plot, $\log \beta_{\text{NiAB}}^{\text{Ni}}$ vs. $1/T$ (Figure 2). The thermodynamic functions (ΔG , ΔH and ΔS) for binary systems are given in Table 3. For binary systems, (i) the ΔH values are negative (except 2abt) indicating the exothermic nature of reaction. (ii) the negative values of ΔG show that the driving tendency of the complexation reaction is spontaneous process. (iii) the ΔS values of the complexation process are positive, confirming that the complexation process is entropically favorable.

All the thermodynamic parameters of the mixed ligand complexes are given in Table 4. The $\log \beta_{\text{NiAB}}^{\text{Ni}}$ increases with increasing temperature and $\log \beta_{\text{NiAB}_2}^{\text{Ni}}$ decreases with increasing temperature (Figure 2). A negative va-

Table 2. Stability constants for the Ni(II)-2abt(A)-gly, ala, val and pal(B) mixed ligand complexes in 50% (v/v) water – ethanol mixture at 300, 310, 320 and 330 K, I = 0.15 M (NaClO₄)

Parameters	Ligand (B) (at 300 K)				Ligand (B) (at 310 K)				Ligand (B) (at 320 K)				Ligand (B) (at 330 K)			
	gly	ala	val	pal												
$\log \beta_{\text{NiAB}}$	10.09(3)	10.26(3)	9.31(4)	9.78(5)	10.48(2)	10.29(2)	10.11(3)	10.03(4)	10.56(2)	10.44(2)	10.38(3)	10.09(4)	10.59(3)	10.57(2)	10.62(3)	10.30(4)
$\log \beta_{\text{NiAB}_2}$	14.68(4)	15.10(5)	14.79(5)	14.44(6)	14.60(4)	14.98(3)	14.38(5)	14.17(6)	14.53(3)	14.81(4)	14.32(4)	14.03(6)	14.13(4)	14.71(4)	14.26(4)	13.95(5)
$\log K_{\text{NiAB}}^{\text{NiA}}$	6.00	6.17	5.84	5.69	6.16	5.98	5.80	5.72	6.00	5.88	5.82	5.53	5.70	5.69	5.73	5.41
$\log K_{\text{NiAB}}^{\text{NiB}}$	4.36	4.59	4.24	4.26	4.81	4.74	4.62	4.59	5.01	5.03	4.96	4.76	5.15	5.26	5.27	5.09
$\log K_{\text{NiAB}_2}^{\text{NiA}}$	10.59	11.01	10.70	10.35	10.29	10.67	10.05	9.86	9.97	10.25	9.76	9.47	9.24	9.83	9.37	9.06
$\Delta \log K_{\text{NiAB}}$	0.27	0.50	0.16	0.17	0.50	0.43	0.31	0.28	0.46	0.47	0.32	0.28	0.26	0.37	-0.08	-0.23
$\Delta \log K_{\text{NiAB}_2}$	0.25	0.45	0.49	0.34	0.23	0.40	0.03	0.07	0.39	-0.23	-0.23	-0.19	-0.30	-0.20	-0.10	-0.27
$\log X_{\text{NiAB}}$	3.41	3.54	3.94	3.11	4.10	3.53	3.38	3.48	4.31	3.64	3.96	3.43	4.44	3.92	4.46	3.95
$\log X_{\text{NiAB}_2}$	2.02	2.25	1.91	1.92	2.33	2.26	2.31	2.11	2.48	2.49	2.34	2.31	2.84	2.95	2.50	2.80

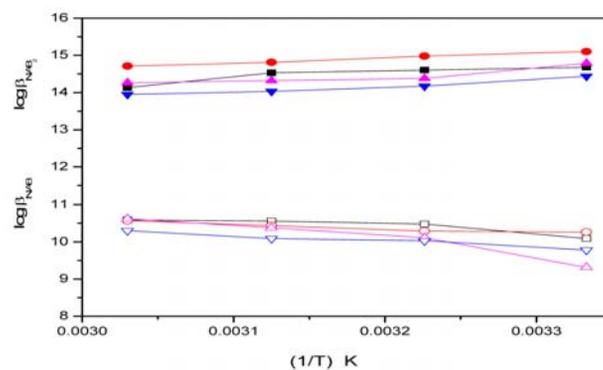


Figure 2. Van't Hoff plot of $\log \beta_{\text{NiAB}}$ and $\log \beta_{\text{NiAB}_2}$ vs $1/T$. [□] Ni(II)-2abt-gly, [O] Ni(II)-2abt-ala, [△] Ni(II)-2abt-val and [▽] Ni(II)-2abt-pal in NiAB species, [■] Ni(II)-2abt-gly, [●] Ni(II)-2abt-ala, [▲] Ni(II)-2abt-val and [▼] Ni(II)-2abt-pal in NiAB₂ species

lue of ΔG for these complexes suggests that the complexation process is spontaneous. A negative value of ΔH in NiAB complexes shows that the process is exothermic and favorable at low temperature. But NiAB₂ complexes show a positive value indicates that the process is endothermic and favorable at higher temperature. All the complexes show positive ΔS values predict that the complexation of 2abt with Ni(II) in presence of amino acids is entropically favorable.

3. 4. Electronic Spectra

All the electronic absorption spectra of the complexes were taken at different pH in 50% (v/v) water-ethanol mixture at 3×10^{-3} M concentration and the values are given in Table 5. The spectral bands of both binary and mixed ligand complexes have low molar absorptivity. This shows that hexa coordination is achieved by required number of water molecules. The spectral data (Table 5) confirms NiAB complexes have distorted octahedral geometry with ${}^3A_{2g}$ as ground state^{24,25}. The values of the ligand field splitting energy (Dq), Racah interelectronic repulsion parameter (B), nephelauxetic ratio (β), percentage of covalency β^o (%) and LFSE for NiAB complexes have been calculated and given in Table 5. The Racah parameter B is less than the free ion value (1041 cm^{-1}) and the β values lies in the range of 0.66 – 0.72 indicating the covalent character of the complexes. The values of ν_2/ν_1 (1.66–1.77) and β^o (28–34%) supports the distorted octahedral geometry²⁵ around the Ni(II) ion.

3. 5. Species Distribution Diagram

Distribution diagram for all the mixed ligand complexes in the present investigation has been obtained for different metal to 2abt and amino acid ratio. The formation of NiAB complex starts at pH 5 and it has been found to be maximum in the pH range of 7.0 to 8.0 and accoun-

Table 3. Thermodynamic parameters of Ni(II)-binary systems

System	Species	–ΔG (kJ mol ^{–1})				ΔH (kJ mol ^{–1})		ΔS (J K ^{–1} mol ^{–1})			
		300 K	310 K	320 K	330 K	300 K	310 K	320 K	330 K		
Ni(II)-2abt	HA	22.63	22.56	22.49	22.43	–24.65	–6.73	–6.76	–6.77	–6.73	
	NiA	23.49	25.58	27.94	30.90	49.88	244.57	243.42	243.18	244.78	
	NiA ₂	36.99	40.30	43.50	45.49	49.33	287.72	289.12	290.09	287.33	
Ni(II)-gly	HA	56.12	56.80	56.37	56.68	–52.44	12.26	14.07	12.27	12.83	
	H ₂ A	69.22	69.68	69.85	70.70	–55.43	45.96	45.98	45.06	46.29	
	NiB	32.92	33.60	33.94	34.37	–18.72	47.31	47.98	47.57	47.43	
	NiB ₂	59.39	59.71	59.56	60.28	–52.02	24.58	24.82	23.55	25.03	
Ni(II)-ala	HA	57.84	57.69	58.08	58.76	–48.50	31.15	29.66	29.95	31.10	
	H ₂ A	71.11	72.71	73.03	73.74	–46.47	82.15	84.66	83.02	82.63	
	NiB	32.57	32.94	33.15	33.55	–23.13	31.45	31.64	31.29	31.57	
	NiB ₂	60.66	60.96	62.13	63.31	–33.34	91.05	89.08	89.96	90.82	
Ni(II)-val	HA	58.07	58.20	58.39	59.43	–44.56	45.06	43.91	43.24	45.35	
	H ₂ A	72.32	73.90	74.57	74.87	–47.14	83.93	86.32	85.71	84.04	
	NiB	32.63	32.59	32.72	33.30	–26.28	21.15	20.34	20.12	21.26	
	NiB ₂	58.59	59.65	59.43	59.96	–22.93	38.67	40.85	38.89	39.32	
Ni(II)-pal	HA	54.86	55.56	56.31	56.61	–36.72	60.45	60.76	61.21	60.28	
	H ₂ A	67.03	68.26	69.05	68.37	–52.35	48.95	51.33	52.20	48.54	
	NiB	31.71	32.29	32.17	32.73	–22.93	29.26	30.19	28.86	29.70	
	NiB ₂	57.50	58.11	58.02	59.01	–44.24	44.20	44.74	43.08	44.78	

Table 4. Thermodynamic parameters of Ni(II)-2abt(A)-amino acid(B) mixed ligand systems

Mixed ligand complexes	Species	–ΔG (kJ mol ^{–1})				–ΔH (kJ mol ^{–1})		ΔS (J K ^{–1} mol ^{–1})			
		300 K	310 K	320 K	330 K	300 K	310 K	320 K	330 K		
Ni-2abt-gly	NiAB	57.97	60.17	64.73	66.91	11.32	230.98	263.48	278.16	289.41	
	NiAB ₂	84.33	86.74	89.05	89.30	–36.86	158.25	205.28	190.20	189.79	
Ni-2abt-ala	NiAB	58.96	61.09	63.96	66.80	20.09	237.08	261.87	278.76	290.97	
	NiAB ₂	86.74	88.90	90.74	92.97	–25.16	160.59	205.59	185.26	187.36	
Ni-2abt-val	NiAB	57.05	57.63	58.69	57.85	26.40	237.67	262.63	278.15	289.06	
	NiAB ₂	84.97	82.59	84.15	81.99	–27.92	163.09	204.93	193.26	187.36	
Ni-2abt-pal	NiAB	56.17	59.55	61.84	65.06	30.66	218.65	244.88	256.39	272.12	
	NiAB ₂	82.94	84.08	85.96	88.16	–26.01	134.31	179.89	163.86	164.08	

Table 5. Electronic absorption spectral data of Ni(II)-2abt(A) and Ni(II)-2abt(A)-amino acid(B) mixed ligand complexes: [M] = [A] = [B] = 3 × 10^{–3} M at 310 K in 50% (v/v) water – ethanol mixture at pH = 8.0

Complex	λ _{max} (cm ^{–1})	Band assignments	Geometry	10 Dq (cm ^{–1})	Ligand field parameter			LFSE kJ mol ^{–1}
					B (cm ^{–1})	β'	β ⁰ (%)	
Ni(II)-2abt	13514	³ A _{2g} (F) → ³ T _{1g} (F)	Octahedral	–	–	–	–	–
	23810	³ A _{2g} (F) → ³ T _{1g} (F)						
	10438	³ A _{2g} (F) → ³ T _{1g} (P)						
Ni(II)-2abt-gly	17301	³ A _{2g} (F) → ³ T _{1g} (F)	Distorted	1044	692	0.66	34	149.94
	24390	³ A _{2g} (F) → ³ T _{2g} (F)	Octahedral					
	10395	³ A _{2g} (F) → ³ T _{1g} (P)						
Ni(II)-2abt-ala	18450	³ A _{2g} (F) → ³ T _{1g} (F)	Distorted	1040	754	0.72	28	149.32
	24038	³ A _{2g} (F) → ³ T _{2g} (F)	Octahedral					
	10549	³ A _{2g} (F) → ³ T _{1g} (P)						
Ni(II)-2abt-val	18382	³ A _{2g} (F) → ³ T _{1g} (F)	Distorted	1055	714	0.69	31	151.53
	23981	³ A _{2g} (F) → ³ T _{2g} (F)	Octahedral					
	10482	³ A _{2g} (F) → ³ T _{1g} (P)						
Ni(II)-2abt-pal	18450	³ A _{2g} (F) → ³ T _{1g} (F)	Distorted	1048	748	0.72	28	150.57
	24213	³ A _{2g} (F) → ³ T _{2g} (F)	Octahedral					
		³ A _{2g} (F) → ³ T _{1g} (P)						

ted ca. 40–80% of the total metal ion. NiAB₂ complex has been found to be maximum favored in the pH 7.0 to 7.5 and accounted ca. 10–20% of the total metal in this form. At lower pH NiA and NiA₂ complexes are present in con-

siderable amount. At higher pH less than 10% of total metal ions are present as NiB and NiB₂ complexes. A representative species distribution diagram is given in Figure 3.

3. 6. Biological Activities

The investigation of antibacterial and antifungal activities (Table 6) shows that the biological activity is found to be in the following order: Control > mixed ligand complexes > Ni(II)-2abt(A). This enhancement in the activity can be explained on the basis of chelation theory²⁶. Chelation reduces the polarity of the metal ion to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Also, it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This enhancement in the activity may also be explained on the basis of their structures by mainly possessing additional electron donor group ($-\text{NH}_2$) present in 2abt²⁷. A representative graph is given in Figure 4.

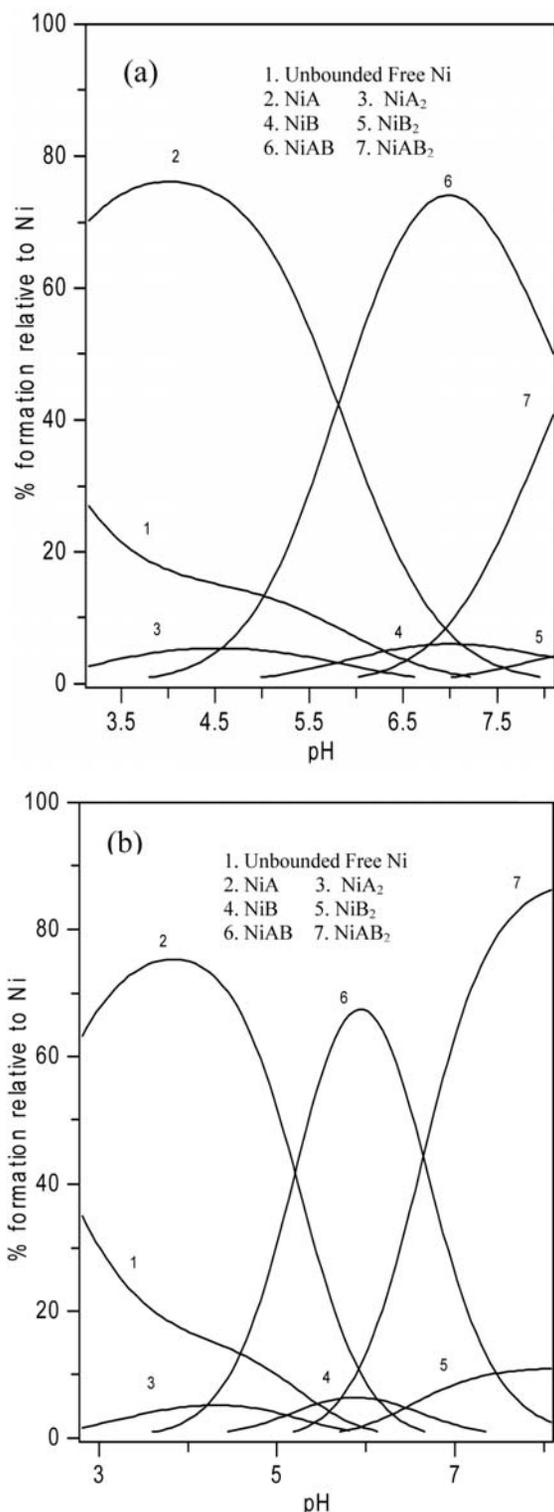
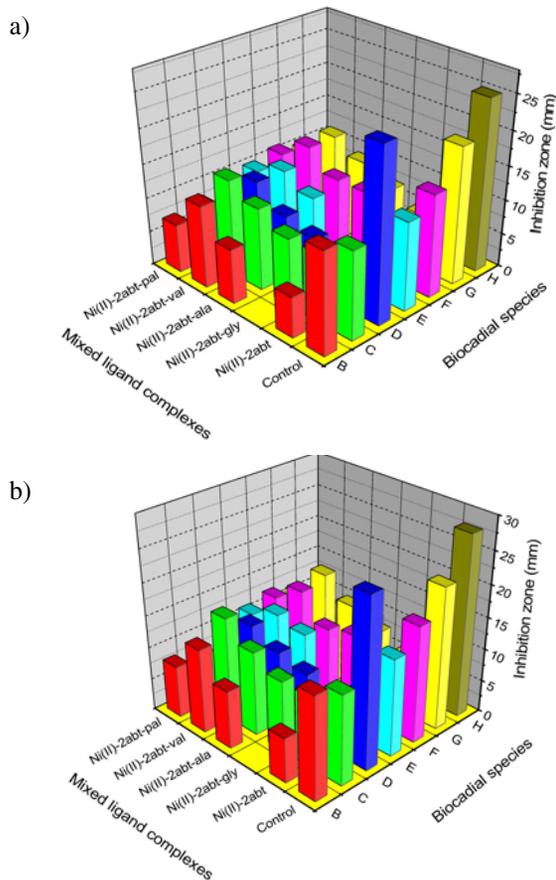


Figure 3. Species distribution diagram for (a) Ni(II)-2abt(A)-val(B) and (b) Ni(II)-2abt(A)-pal(B) mixed ligand complex system, at $C_M = C_A = 0.003$ and $C_B = 0.006$ mol dm⁻³ at 310 K.



B – *Salmonicine aureus* C – *Pseudomonas aeruginosa* D – *Escherichia coli* E – *Aspergillus niger* F – *Penicilline species* G – *Tricoderma virida* H – *Saccharomyces species*

Figure 4. Biological activities of Ni(II)-2abt(A) and Ni(II)-2abt(A)-amino acid(B) mixed ligand complexes (a) at 24 h and (b) at 48 h by well diffusion method (zone formation in mm)

Table 6. Biological activities of binary and mixed ligand complexes by well diffusion method (zone formation in mm)

Complexes	Diameter of inhibition zone in mm for different microbial species													
	Sal		Pseudo		E. Coli		A. Niger		Penicilline		Triv		Saccharo	
	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	Time (h)	
Control	15	16	13	14	25	26	13	15	15	18	20	22	25	28
Ni(II)-2abt	6	7	10	11	–	–	6	7	–	–	8	9	–	–
Ni(II)-2abt-gly	–	–	10	11	8	10	–	–	11	12	10	11	–	–
Ni(II)-2abt-ala	8	9	12	13	9	11	10	12	11	11	12	13	7	9
Ni(II)-2abt-val	12	13	14	16	12	13	12	13	14	15	14	16	8	9
Ni(II)-2abt-pal	7	8	8	8	10	10	10	11	11	12	10	11	–	–

3. 7. DNA Cleavage Activity

In the present study, the oxidative CT DNA cleavage activity of Ni(II)-2abt (A) and Ni(II)-2abt(A)-gly/ala/val/pal(B) complexes at 37 °C was studied by gel electrophoresis in presence of oxidant H₂O₂ (Figure 5). The DNA cleavage efficiency of the complex was due to the different binding affinity of the complex to DNA. Control experiment using DNA alone (lane 1) does not show any significant cleavage even on longer exposure time. From the observed results, it is clear that the Ni(II)-2abt (lane 2) cleave DNA as compared to control DNA. Probably this may be due to the formation of redox couple of the metal ion and its behavior²⁸. It is also thought that, most cleavage cases are caused by nickel ions reacting with H₂O₂ to produce the diffusible hydroxyl radical (–OH) or molecular oxygen, which may damage DNA through Fenton type chemistry.

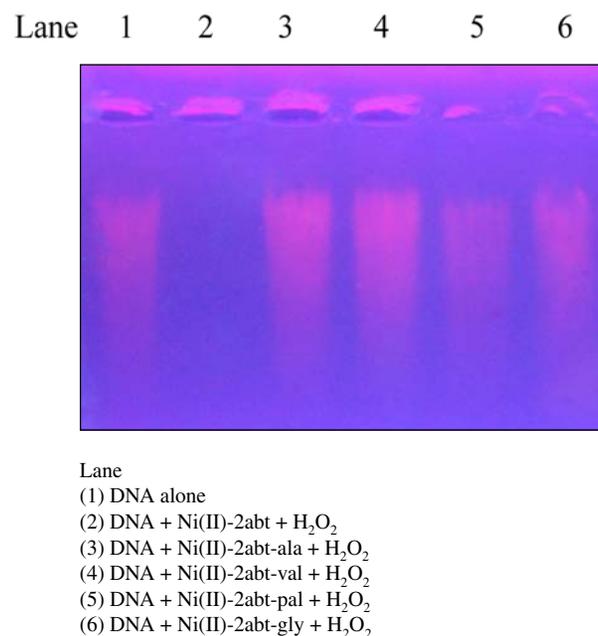


Figure 5. Changes in agarose gel electrophoretic pattern of calf-thymus DNA induced by mixed ligand complexes in presence of H₂O₂

4. Conclusion

In the present work, we determined the stability constants of binary and mixed ligand complexes in varying temperatures. The percentage distribution of various binary and mixed ligand species in solution was evaluated. The stabilization parameters calculated demonstrates that the complexation of 2abt with Ni(II) ion in presence of amino acid is more favorable than the binary complex formation. The thermodynamic functions calculated explain the nature of the complexation process. The pH-metric and electronic spectral studies predicts distorted octahedral structure for all the studied complexes. The mixed ligand complexes show more potent antimicrobial activity against microorganisms and cleave CT DNA in the presence of H₂O₂.

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Povzetek

V temperaturnem območju med 300 in 330 K smo potenciometrično raziskovali kompleksacijo 2-aminobenzotiazola (2abt) [A] z Ni(II) v prisotnosti različnih amino kislin; glycina (gly), L-alanina (ala), L-valina (val) ter L-fenilalanine (pal) [B] v 50 % (v/v) mešanici vode in etanola, ki je vsebovala 0.15 M NaClO₄. Predpostavili smo nastanek kompleksov tipa NiAB in NiAB₂ ter jim določili konstante stabilnosti. S pomočjo temperaturne odvisnosti konstant stabilnosti smo ocenili termodinamske parameter (ΔG , ΔH and ΔS) za procese nastanka kompleksov.

Lastnosti kompleksov smo raziskovali tudi s pomočjo elektronskih spektrov ter ugotovili, da imajo oktahedralno strukturo. Proučevali smo tudi možno mikrobiološko aktivnost *in vitro* na nekaterih bakterijah, glivah in kvasovkah ter njihov vpliv na cepitev DNA.