Spectroscopic Study of Hydrazone-based Ligands as Calorimetric Reagents for Acetate Anion

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A B S T R A C T

This study focuses on investigating the chromogenic properties of hydrazone compounds derived from the reaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with salicyaldehyde and 2-hydroxyacetophenone for anion recognition. Two chromogenic ligands, 2-[[2-(2,4-dinitrophenyl)hydrazone]methyl]phenol (L1) and 1-(2-hydroxy-phenyl)-ethanone-(2,4-dinitro-phenylhydrazone) (L2), were synthesized and characterized using IR and NMR spectroscopy. The complexation abilities of these ligands towards various anions (acetate, chloride, carbonate, nitrate, fluoride, iodide, sulphite) were investigated using UV-Vis spectroscopy. Both ligands showed higher selectivity towards the acetate anion in DMSO compared to the other anions. The complexes exhibited maximum absorptions (λ_{max}) at 502 nm and 499 nm for L1 and L2, respectively. The Job plot results showed a 1:1 stoichiometry between the ligands and the acetate anion. The stability constants for both complexes were found to be 3.77×10^{-8} and 9.0×10^{-8}, respectively. No significant interference from other anions on the complexes was observed.

1 Introduction

Hydrazones are organic compounds consisting of hydrazine (N_2H_2) interaction with carbonyl compounds in acid solution [1]. It is characterized by the presence of the group of zomithins (-NH-N = CH-) in its molecules [2]. These compounds are important links that possess interesting union characteristics due to the existence of multiple coordination sites [3]. There relative readiness makes it attractive to design new melting compounds that include this part [4]. Dinitrophenylhydrazones have an acid group of N-H that can generate anions with the ability to donate electrons. This connects nitrogen anion to an acceptance group to interesting pigments[5].

The importance of identifying and sensing anions is a great concern for their crucial role in the biological, industrial, medical and environmental fields. Where, a great effort has been focused on the innovation and manufacture of selective ion receptors, where the identification of anion becomes one of the fastest growing branches of supramolecular chemistry [6]. Ion selective attracts eye sensing becomes a particular challenge, because optical detection provides immediate qualitative information, while absorption spectroscopy gives quantitative information. Most chromophore sensors are collectively linked to the anion identification unit. However, there are only a few colorimetric sensors identified to measure the presence of anionic substrates [7]. Among different types of anions, carboxylate anions are biochemically important because they play vital roles in enzymes, antibodies and many metabolic processes [8]. Identifying acetate anion

and its sensitivity is more important than other biological functional anions. Increase their biological importance, as acetate also plays larger roles in chemical industries such as plastic, pharmaceuticals and food. There are many shortcomings in the literature about anion sensitivity, where some studies are limited to non-competitive organic solvents [9]. This limitation may be due to competitive interactions between primary solvents and anions through hydrogen interconnectedness. Therefore, the recognition of acetate anion in aqueous solution is very difficult [10.11]. This paper is interested in studying the colorimetric behaviour of hydrazone compounds when complexed with acetate anion

Materials and Methods

Instruments

Infrared spectra of formed compounds were recorded using Perkin-Elmer 2000 FT-IR spectrum device. Potassium bromide pellets (KBr) were used and the frequency range was 4000-400 cm^{-1}. ^{1}H NMR spectra were recorded on the Bruker 500 MHz NMR spectrometer, and chemical transformation values were in ppm using DMSO-d6. Basic elemental analysis (CHN) was performed using the CHN/O-Perkin Elmer Series II 2400. The UV spectrometers were registered according to the UV/Vis Perkin-Elmer-Lambda-25 spectrometer, the range was from 800-200 nm. 1.0cm using Quartz Cell.

Chemicals and Reagents

The chemicals and reagents used this work were analytical grade and were used without any treatment. The following chemicals were used: 2, 4-dnitrophenylhydrazine (DNPH) (BDH), salicylaldehyde, and 2-hydroxyacetophenone (Fluka).

Synthesis of Hydrazone Compounds

Synthesis of 2-[(2-(2,4-dinitrophenyl)hydrazone)-methyl]phenol, (**L1**)

To a clear solution of DNPH (3.96 g, 0.02 M) in 60 mL of ethanol containing 15 mL of concentrated H$_2$SO$_4$, add 30 mL of an equimolar ethanolic solution of salicylaldehyde (2.44 g, 0.02 M) with continuous stirring. After a few minutes, an orange-yellow solid precipitate will form. Filter the precipitate using a

Buchner funnel and wash with ethanol. Dry the precipitate in the oven at 50°C. The yield obtained was 80%, with a melting point of 184-188°C. IR (KBr): v(N-H) = 3273 cm^{-1}, v(OH) = 3373 cm^{-1}, v(C=N) = 1620 cm^{-1}, v(NO$_2$) = 1331 cm^{-1}, v(C-H) = 3100 cm^{-1} (aldehyde), v(C=C) = 1587-1518 cm^{-1} (aromatic). ^{1}H NMR (500 MHz, DMSO-d$_6$): = 11.2 ppm (s, 1H, NH), 10.2 ppm (s, 1H, OH), 8.90 ppm (s, 1H, N=CH), 6.90-8.85 ppm (m, 7H, aromatic CH). CHN analysis for C$_{13}$H$_{10}$N$_4$O$_5$ (302.24): calculated; C 51.61, H 3.31, N 18.50; found C 50.11, H 3.26, N 17.87.

Synthesis of 1-(2-hydroxy-phenyl)-ethanone-(2,4-dinitro-phenylhydrazone), (L2)

To a clear solution of DNPH (prepared as above), add 30 mL of an equimolar ethanolic solution of 2-hydroxyacetophenone (2.72 g, 0.02 M) with continuous stirring. After a few minutes, a red solid precipitate will form. Filter the precipitate using a Buchner funnel and wash with ethanol. Dry the precipitate in the oven at 50°C. The yield obtained was 80%, with a melting point of 168-173°C. IR (KBr): v(N-H) = 3299 cm^{-1}, v(OH) = 3400 cm^{-1}, v(C=N) = 1618 cm^{-1}, v(NO$_2$) = 1334cm^{-1}, v(C-H) CH$_3$ = 1421cm^{-1} (bending), v(C=C) = 1597-1518cm^{-1} (aromatic). ^{1}H NMR (500 MHz, DMSO-d$_6$): = 11.1 ppm (s, 1H, NH), 10.75 ppm (s, 1H, OH), 8.95 ppm(s, 1H, N=CH), 6.90-8.45 ppm (m, 7H, aromatic CH), 2.50 ppm (s, 3H, CH3). CHN analysis for C$_{13}$H$_{10}$N$_4$O$_5$ (316.27): calculated; C 53.12, H 3.79, N 17.71; found C 52.91, H 2.20, N 16.58.

Spectrophotometric Analysis

Stock solutions (1.0×10^{-4} M) of each ligand (0.0015g, **L1**) and (0.0158g, **L2**) were prepared in 50 mL of DMSO and stored at room temperature. A 1.0×10^{-4} M stock solution of different anions was also prepared by dissolving the appropriate amount of various anions in 10 mL of DMSO. Equal mole ratio mixtures (1:1, ligand: anion) were prepared by diluting the stock solutions accordingly. The reagents were added in the following order: ligand–anion. Then, the volume was diluted to the mark with the corresponding DMSO solvent in a 10 mL volumetric flask. Finally, the absorbance of the solutions was measured spectrophotometrically.

2   Results and Discussion

Synthesis and Characterization

The ligands were prepared according to the Vogel method [12]. Ligand 1 (L1) was manufactured according to one DNPH equivalent intensification method with one salicylaldehyde equivalent, and ligand 2 (L2) was prepared with one DNPH equivalent intensification method with the equivalent of 2-hydroxyacetophenone. The synthetic methods of preparation of L1 and L2 are described in figure 2.
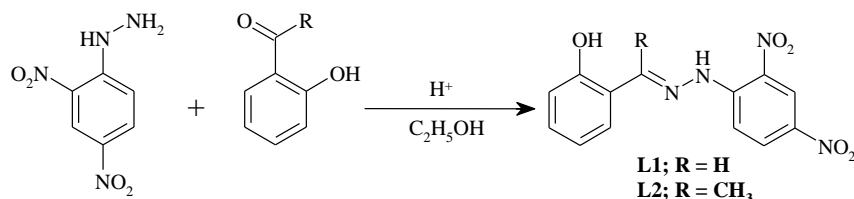
Synthesised compounds have shown good solubility in common organic solvents such as THF, DMSO, $CHCl_3$, MeCN and acetone at room temperature. However, they showed only moderate solubility in polar protic solvents like methanol and ethanol, and were practically insoluble in water.

Figure 1: The synthetic routes for the preparation of **L1** and **L2**.

Chromogenic Properties of L1 & L2 ligands
Solvatochromic Properties
The UV-visible spectra of the hydrazone compounds were recorded in various solvents. Since both ligands were insoluble in water, different common organic solvents ($CHCl_3$, CH_3CN, CH_3COCH_3, DMSO, THF, C_2H_5OH, and CH_3OH) were used to investigate the effect of these solvents on the chromogenic properties of the ligands. The λ_{max} and absorption values for both

ligands in different solvents are provided in **Table 1**. The main absorption bands were observed in the visible region, ranging from 377 to 407 nm, which can be attributed to the π-π* transition in the large conjugated system. This transition is associated with intramolecular charge transfer. DMSO was found to be the most suitable solvent, showing higher absorption for both ligands. This could be attributed to the solubility of both ligands in relatively polar solvents.

Table 1: UV-Vis characteristics of L1 and L2 in different solvents.

Solvent	Dielectric constant	L 1		L 2	
		λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance
MeOH	33.0	382	0.612	379	0.325
EtOH	24.5	382	0.551	377	0.321
Acetone	21.0	382	0.94	381	0.403
THF	7.5	381	1.02	380	0.403
DMSO	18.4	407	1.33	382	0.853
MeCN	37.5	382	1.061	381	0.491
$CHCl_3$	4.8	381	0.613	386	0.449

Methanol, MeOH; Ethanol, EtOH; Acetonitrile, MeCN.

Complexation with anions
Since the ligands showed high absorption values in DMSO, the complexity properties of these compounds were carried out with several anions using DMSO as the medium of complexity. Figure 2 shows the absorption spectrum of each ligand (1×10^{-5}M) in DMSO when adding quantitative equivalent to certain anions (NO_3^- CH_3COO^- and Cl^-). No change was observed in the color of the solution when these anions were added, except for acetate anion (OAc^-).

Adding the OAc^- anion to both ligands separately resulted in an immediate change in color from yellow to red. New absorption bands at λ_{max}, 501 and 491 nm for both ligands complexes (respectively) were observed, indicating that complex formation had taken place. Since OAc^- was the only anion that showed a clear change in color upon complexation with both ligands, it was selected for further studies.
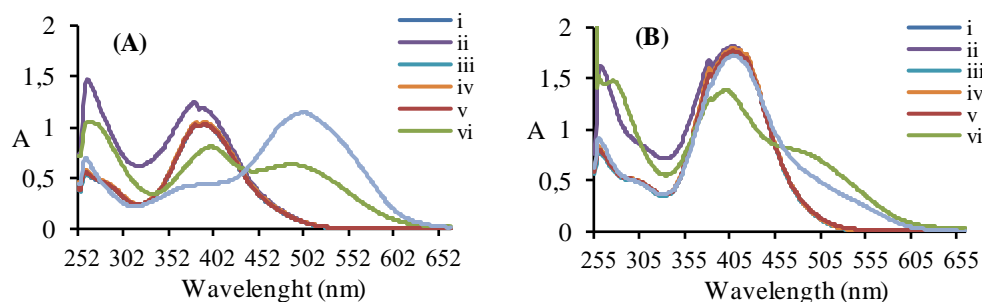
Figure 2: The absorption spectra of 1×10^{-5}M of (A) **L1** and (B) **L2** in DMSO upon addition of equimolar amounts of various anions: (i) $AgNO_3$, (ii) $Fe(NO_3)_3$, (iii) $LiNO_3$, (iv) $Ni(NO_3)_2$, (v) $CoCl_2$, (vi) $Cu(CH_3COO)_2$, (vii) $Pb(CH_3COO)_2$.

The effect of the cation on the chromogenic behavior of both ligands towards the acetate anion was also investigated. Therefore, the study was further carried out using different sodium salts, namely acetate, chloride, carbonate, nitrate, fluoride, iodide, sulphite, and bromide. Both ligands showed very good selectivity towards the acetate anion (**Figure 3**), with the color of the solutions changing from yellow to red immediately upon the addition of the acetate solution, while no changes were observed with other anions. The color responses for the selected anion could arise from the basicity of the anion [13].
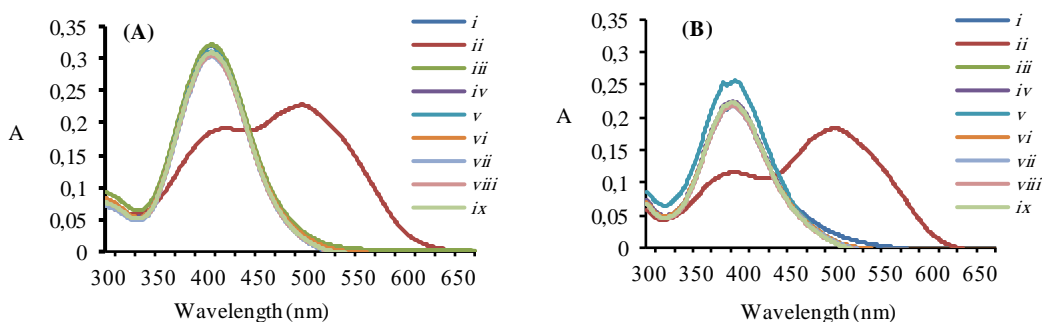

Figure 3: The absorption spectra of 1×10^{-5}M of (A) **L1** and (B) **L2** in DMSO upon addition of equimolar amounts of different sodium salts, (*i*) ligand, (*ii*) CH_3COO^-, (*iii*) Cl^-, (*iv*) Br^-, (*v*) F^-, (*vi*) I^-, (*vii*) NO_3^-, (*viii*) CO_3^{2-}, (*ix*) SO_3^{2-}

Effect of ligand concentration

Optimal ligand concentration is another important factor affecting the complexity of ligands using OAc^-. Therefore, the effect of binding concentrations on the absorption of their compounds with OAc^- was studied by mixing fixed doses (1.0×10^{-5} M) of OAc^- anion and different concentrations of ligands, varying from 8×10^{-7} to 8.0×10^{-5} M in DMSO (Figure 4). It was observed that the severity of the color of the test solution increased as the ligand concentration increased. The absorption was found to be proportional to the ligand concentration in the range from 2×10^{-6} to 2.0×10^{-5} M and 2×10^{-6} to 1×10^{-5} M for the complexes L1-OAc$^-$ L2-OAc$^-$, respectively (Figure 5). Absorption was maximized when the concentration of ligands doubled, compared to the concentration of OAc^- by mole-ratio 2:1 for both ligands, as this ratio was very necessary to obtain the most appropriate development of colors and to maximize absorption. After this ratio, it was observed that absorption decreased with an increase in the concentration of ligands.
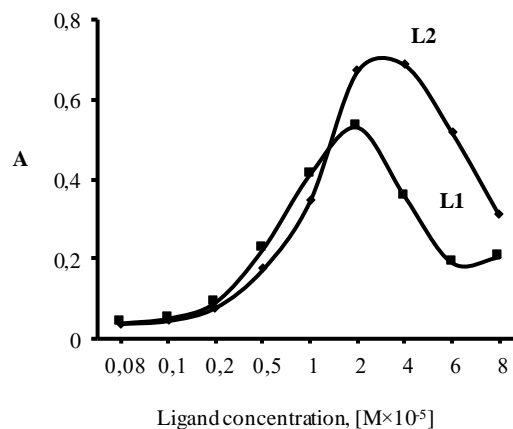

Figure 4: Effect of L1 (502 nm) and L2 (499 nm) concentration on their complexation with acetate anion (1.0×10^{-5}M) in DMSO.
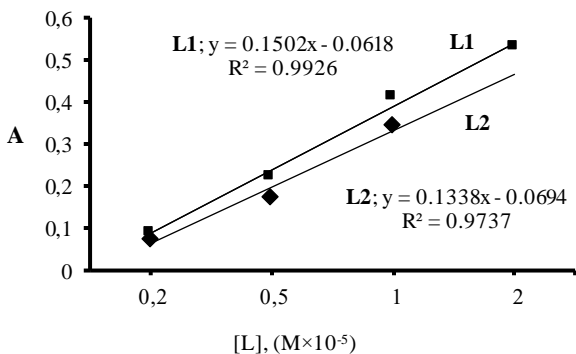
Figure 5: Plot of absorbance of **L1**-OAc⁻ and **L2**-OAc⁻ complexes in DMSO.

Effect of acetate concentration

At a fixed ligand concentration of 2.0×10^{-5}M for both ligands, we investigated the effect of the OAc⁻ anion. To examine the absorption profile as a function of OAc⁻ concentration, we varied the total amount of OAc⁻ anion in the reaction mixture from 8×10^{-7} to 8.0×10^{-5} M. The maximum absorbance of the ligand-OAc⁻ complex (Figure 6) was observed at 2.0×10^{-5} M of OAc⁻ anion with a molar ratio of 1:1 (ligand:OAc⁻). We noticed that increasing the OAc⁻ concentration did not significantly decrease the absorbance of both complexes. The absorbance was found to be linearly proportional to the concentration of OAc⁻ in the range of 1.0×10^{-6} to 1×10^{-5}

M and from 1.0×10^{-6} to 2×10^{-5} M for both complexes, respectively. Thus, we used an acetate concentration of 2×10^{-5} M for further studies. The appearance of isosebestic points (Figure 7) indicated that stable complexes were formed with a specific stoichiometric ratio between ligands and acetate anion [14]. These findings confirm the complex formation between acetate and both ligands, and suggest that the stoichiometry of the reaction remains unchanged during the chemical reaction at a specific wavelength. The total absorbance of the chemical species, including the free ligand and ligand-OAc⁻ complex, remains in equilibrium and does not change during a chemical reaction.
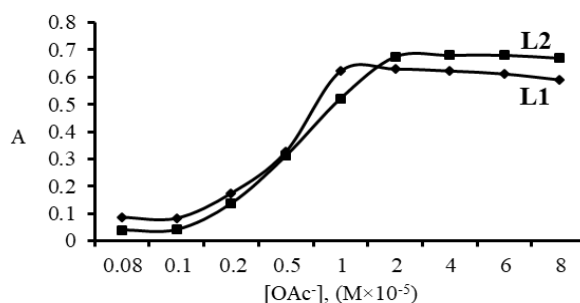

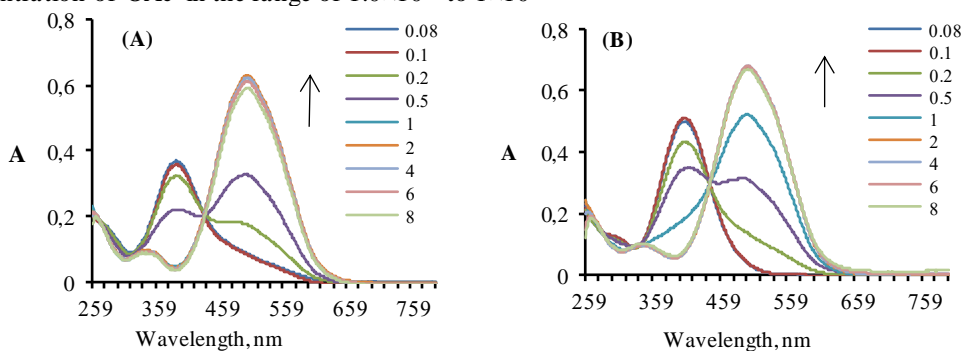**Figure 6:** Effect of acetate concentration on the absorbance of L1-OAc⁻ and L2-OAc⁻ complexes in DMSO.

Figure 7: Changes in absorption spectra as a function of [OAc⁻] in DMSO (**A**) L1= and (**B**) L2 (M= 2×10^{-5} M for both ligands).

Stoichiometry of the complexes

We studied the stoichiometry of both complexes using Job's continuous variations method [15,16]. In the L1-OAc⁻ and L2-OAc⁻ anion complexes, the curve (**Figure 8**) displayed a formation constant of a 1:1 molar ratio. This is consistent with the results of the mole ratio previously mentioned regarding the effect of acetate concentration. The extrapolated value A_{ext} at the point of intersection on the plot corresponds to the total absorbance of the complex, indicating that complex formation has been completed.

The stability constants for both complexes were determined from the plot of Job's method using the following equation.

$$K_s = \frac{1-\alpha}{\alpha^2 C}$$

The calculated values for the stability constants (Ks) of both complexes were found to be 3.77×10^{-8} and 9.0×10^{-8}, respectively. Where = Em-Es (found from graph), C is the concentration of acetate anion.
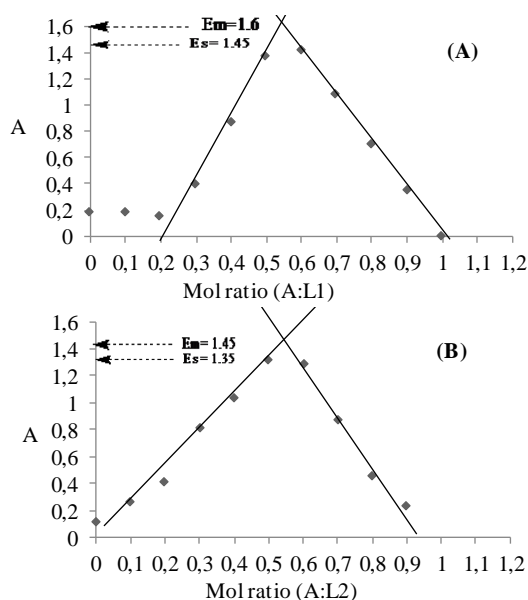
Figure 8: Determination of A:L ratio of (A): L1-OAc⁻, complex, and (B) L2-OAc⁻, complexes by Job's method

Both ligands have an azomethine ($-N=C-$), NO$_2$, and OH groups, which means they could potentially form chromogenic complexes with acetate anion. Hydrazone derivatives are known to selectively interact with fluoride and acetate anions through hydrogen bonding due to the basicity of anions [7,13,17].

Interferences.

The study investigated the interference of foreign anions on the complexation of OAc⁻ with both ligands. A fixed amount (1.0×10^{-5}M) of OAc⁻ anion was added to the ligands solution (1.0×10^{-5}M) in the presence of one equivalent of other anions (i.e., Cl⁻, NO$_3$⁻, I⁻, F⁻, CO$_3$²⁻, SO$_3$²⁻, and Br⁻). **Table 2** shows the change (%I) in the absorbance of both ligands upon the addition of other anions. No remarkable interferences on the complexation of OAc⁻ with both ligands were reported.

Table 2: The interferences of foreign anions.

Anion	L1			L2		
	A	ΔA	%I	A	ΔA	%I
OAc⁻	0.4004	-	-	0.9154	-	-
OAc⁻ + CO$_3$⁻²	0.4074	0.007	1.75	0.9274	0.012	1.31
OAc⁻ + Br⁻	0.4071	0.0067	1.67	0.9329	0.0175	1.91
OAc⁻ + Cl⁻	0.3991	-0.0013	-	0.9218	0.0064	0.70
OAc⁻ + F⁻	0.4036	0.0032	0.80	0.9306	0.0152	1.66
OAc⁻ + I⁻	0.4109	0.0105	2.62	0.9503	0.0349	3.81
OAc⁻ + NO$_3$⁻	0.412	0.0116	2.89	0.9326	0.0172	1.88
OAc⁻ + SO$_3$⁻²	0.4066	0.0062	1.55	0.9173	0.0019	0.21

$\Delta A = A_{(OAc^- + foreign\ anions)} - A_{(OAc^-)}$, %I = $(\Delta A/(A))\times100$

4 Conclusion

In summary, we have succeeded in manufacturing two hydrazon compounds 2-((2-(2,4-dinitrophenyl)hydrazine)methyl)- phenol (L1) and 1-(2-hydroxy-phenyl)-ethanone-(2,4-dinitro-phenylhydrazone) (L2). The study examined the colorimetric complexity characteristics of both ligands towards anion ~~bonds~~ (Cl⁻, Br⁻, I⁻, F⁻, NO$_3$⁻, CO$_3$²⁻, SO$_3$²⁻ and CH$_3$COO⁻). Both ligands showed selectivity towards acetate anion in DMSO. When adding OAc⁻ anion to a solution containing L1 or L2, a clear change in color from yellow to red was immediately observed with the naked eye. The L1-OAc⁻ complex showed the highest absorption at 502nm, while the L2-OAc⁻ complex showed the highest absorption at 499nm. Both ligands showed a 1:1 molar ratio with acetate. Stability constant (Ks) of 3.77×10^{-8}, 9.0×10^{-8} were reported for L1 and L2, respectively. Table 3 summarizes all the analytical parameters involved in complexation of both ligands with acetate anion in DMSO.

Table 3: Summary of analytical parameters of both complexes in DMSO.

Parameters	L 1	L 2
λ_{max} (nm)	502	499
Beer's law limit ($\mu g\ ml^{-1}$) (M×10^{-5})	0.1-1.0	0.1-2.0
Molar absorptivity (L mol^{-1} cm^{-1})	8×10^{-7}	3.37×10^{-6}
Limit of detection ($\mu g\ ml^{-1}$)	0.007	0.012
LOQ ($\mu g\ ml^{-1}$)	0.02	0.04
Regression equation	Y=0.1768x-0.1404	Y=0.165x-0.1576
Slope (b)	0.1768	0.165
Intercept	0.1404	0.1576
(R²)	0.9361	0.9875
(K_s)	3.77×10^{-8}	9.0×10^{-8}

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