Synthesis, structural elucidation and antimicrobial activities of 5-(3-nitrophenyllazo)-6-aminouracil and its complexes with some transition metal ions

Z M Zaki¹, S M Abbas¹, H A Dessoukii ², H S Awes¹, Rehab Mahmoud³*

¹ Inorganic Chemistry Lab. Department of Chemistry, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.
² chemistry department, faculty of science, Benha university, Egypt
³ Materials Science Lab, Chemistry Department, Faculty of Science, Beni -Suef University, Beni Suef, Egypt

Abstract. In this study, 5-(3-nitrophenyllazo)-6-aminouracil was prepared by linking 3-nitro aniline to 6-aminouracil. The (1:1) complexes of this ligand with Fe³⁺,Co²⁺,Ni²⁺, Cu²⁺ and Zn²⁺ were prepared and characterized by elemental analysis for C, H and N, thermal analyses (TGA and DTA), FT-IR, ¹H NMR. The molecular weight of the ligand was determined by mass spectral measurement and its fragmentation pattern was reported. The metal content of the complexes was determined by EDTA complexometric titrations and the coordination number of metal in complexes was confirmed by magnetic susceptibility measurements. Antimicrobial activities for ligand and its complexes were studied. The IR and ¹H NMR studies confirmed that 5-(3-nitrophenyllazo)-6-aminouracil behaves as monobasic bidentate ligand bonding to the metal ion through both NH₂ group and N=N groups forming six membered ring and The magnetic moments measurements indicated octahedral geometry for Fe³⁺ complex, tetrahedron structure for Co²⁺, Ni²⁺, Cu²⁺ complexes and square planar geometry for Zn²⁺ complex. Antimicrobial activity measurements indicated that Co²⁺ and Ni²⁺ complexes are biologically active towards bacteria gram (-ve), gram (+ ve) and fungi, while Zn²⁺ complex showed high resistance towards gram (+ ve) bacteria and medium resistance towards gram (-ve).

Keywords: Uracils, synthesis, Uracil azo dyes, azo dye complexes, transition metal complexes, mass spectra, IR, NMR, Thermal analysis, biological activity

1. Introduction

The compounds containing the functional group R-N=N-R⁻ are known as azo compounds. Both R and R' may be either aryl or alkyl radicals forming different colors due to mobility of labile proton across the molecule through a conjugated system where the proton remains in association with intra molecular hydrogen bonding between donor –acceptor atoms through aromatic moietytes [1–3] and
have many application dependent on the photo physical properties of azo hydrazone tautomerism that used in dyes of surfactant micelles and textile fibers [4,5], photographic systems [6], dyeing protein [7,8] and bleaching [9]. The diazonium salts are stabilized by resonance and react as an electrophile with an electron rich coupling component like phenols and anilines through an electrophilic aromatic substitution mechanism. Some heterocyclic azo compounds are used as ligands to generate a special category of metal azo complexes which are exploited enormously in the manufacture of colorimetric sensors [10]. 6-amino uracil has different reactive sites, therefore has different binding modes so, it can coordinate through one of pyrimidine ring nitrogen, the C=O or NH$_2$ [11,12]. It is used as a starting material for synthesis of heterocyclic frameworks of biological significance such as pyrido-pyrrolo and pyrimidopyrimidines [13–15].

Synthesis and study of metal complexes of heterocyclic compounds have wide range of applications [16] especially the compounds containing nitrogen and sulfur as donor atoms whose anticancer and antiviral activities [17] The Antimicrobial activity is one of the most important activities for exploring novel antimicrobial agents that control the resistant pathogens due to overuse of antibiotics. An example for gram negative bacteria is *Escherichia coli*, which is one member of the normal flora in the intestine. Under stress *E.coli* a bacterium become pathogenic and causes severe intestinal, urinary, biliary and abdominal infections. Other than *E.coli* pathogenicity t produces potent and sever toxins very harmful and dangerous for the small intestinal wall [18,19].

![Scheme 1](image)

**Scheme 1.** 5-(3-nitrophenyllazo)-6-aminouracil

On the other hand, *Staphylococcus aureus* is a gram-positive bacterium which is normal inhabitant in the respiratory tract and on the skin. *S. aureus* at skin infection become pathogenic and causes sinusitis, and food poisoning besides producing virulence factors such as potent protein toxins [20].

In this study, metal complexes of 5-(3-nitrophenyllazo)-6-aminouracil with Fe(III), Co(II), Ni(II) and Cu(II) were prepared and subjected to many tools including elemental analysis for C, H and N, thermal analyses (TGA and DTA), FT-IR, $^1$H NMR. to confirm their structure. The study excluded chelation either through N(3) and C(2)=O, or through N(3) and C(4)=O due to hydrogen bonding [21–25] and revealed that the ligand coordinates to the metal cations in bidentate fashion by coordinating to amino group (deprotonated N6) of uracil ring and azo group. The ligand and complexes were subjected to antimicrobial activity investigation against pathogenic *E.coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* as Gram-negative bacteria while *S. aureus* and *S.mutans* as Gram-positive bacteria but *candida albicans* for antifungal evaluation for efficacy enhancement and decreasing the resistance.

2. Materials and method

2.1. Materials and reagents

All chemicals used are of the highest purity grade and the solvents used were spectroscopic grade. 5-(3-Nitro phenylazo)-6-aminouracil was prepared by using 3-nitro aniline, Sodium nitrite and 6-
aminouracil that were purchased from MERCK-Schuchardt, SDFCL-INDIA and EUROMEDEX FRANCE and hydrochloric acid (HCl) were used from Carlo Erba reagent NaOH Piochem for laboratory chemicals, EGYPT, While Metal Chloride salts like: FeCl$_3$, 6H$_2$O, CoCl$_2$.6H$_2$O, NiCl$_2$.6H$_2$O, CuCl$_2$.6H$_2$O and ZnCl$_2$.6H$_2$O were purchased from Aldrish chemical company.

2.2 Instrumentation

The infrared spectral data of the ligand and their complexes were taken in Potassium bromide disc using Perkin Elmer spectrophotometer serial number 1341 covering frequency range 400-4000 cm$^{-1}$. Proton nuclear magnetic resonance ($^1$HNMR) spectra of the ligand and its complexes were determined with a Bruker FT-NMR instrument at 400.1324 MHz in DMSO-d$_6$ with TMS as internal standard spectrometer at NMR laboratory at faculty of pharmacy at Beni-Suef University. Elemental analyses for C, H and N were recorded by Vario EL III Germany Laboratory. Mass spectra were determined on GC MS-Qp1000EX Shimadzu and thermal analysis (TG-DTG) (20-1100°C) was performed with heating rate of 10°C min$^{-1}$ and recorded on Shimadzu thermogravimetric analyzer (TGA-50H) and (DTG-60H) at Microanalytical unit of Cairo university. Magnetic susceptibility measurements were carried out on powder samples and were evaluated at room temperature using the GOUY Method that carried out on Johnson Matthey magnetic susceptibility balance at central laboratory of chemistry department while the Antimicrobial activities of ligand and its complexes were examined at biochemistry central laboratory of chemistry department at Cairo University.

2.3. Preparation of 5-(3-Nitrophenylazo)-6-aminouracil

5-(3-Nitro phenylazo)-6-aminouracil was prepared by diazotization of 3-nitro aniline 0.04 mole (5.5 g) dissolved in 6 mL of concentrated HCl and 30 mL of water (26). The solution was cooled down in a salt/ice bath, and then a cold solution of NaNO$_2$ 0.04 mole, (2.76 g) in 3 mL of water was added in a dropwise manner with constant stirring. The resulting diazonium salt was cooled in a salt/ice bath and then, 0.04 mole (5 g) of 6-Aminouracil dissolved in 25 (ml) (2N) NaOH solution and cooled in salt/ice bath was added to the cold diazonium solution in a dropwise manner with constant stirring. The solution was stirred at 0-5°C for 1 h. and the pH of the reaction mixture was maintained at 4.6 by the simultaneous addition of 2ml of glacial acetic acid. The resulting precipitate was filtered off, washed with cold water and ethanol and dried. Recrystallization from mixture containing (150 ml ethanol, 150 bidistilled water and 2ml of 25% aqueous ammonia solution [26].

The characterization data are listed in Table 1 which shows a good agreement between calculated and found data for the elemental analysis of the prepared compound.

2.4. Preparation of solid 5-(3-Nitro phenylazo)-6-aminouracil complexes

The complexes were prepared by taking 0.01 mole of the metal chloride salt in 5ml of mixture (1:1) aqueous ethanol and heated to 60°C. The precipitated complex was filtered off, washed several times by ethanol (95%) and dried in the oven. The physical properties of all complexes were listed in table 1.

2.5. Metal analysis

Metal content in the complexes was determined by digesting accurate weight (0.03 gm.) of complex in concentrated aqua regia solution (HCl+HNO$_3$) then titrimetric with standard EDTA (0.01M) using suitable indicator [27,28].
Table 1. Analytical data and physical properties of the 5(3-nitrophenylla)azo-6-amino uracil ligand and its prepared complexes

|        | Ligand      | Fe³⁺ | Co²⁺ | Ni²⁺ | Cu²⁺ | Zn²⁺ |
|--------|-------------|------|------|------|------|------|
| M. wt. | 276         | 400.84 | 368.93 | 404.69 | 409.54 | 375.41 |
| M. F   | C₁₀H₆N₆O₄  | C₁₀H₁₆N₆O₄ | C₁₀H₁₆N₆O₄ | C₁₀H₁₆N₆O₄ | C₁₀H₁₆N₆O₄ | C₁₀H₁₆N₆O₄ |
| Color  | Canary yellow | Brownish yellow | Brownish yellow | Brown | Yellow |
| % C    | 43.47%      | 29.93% | 32.52% | 29.56% | 29.30% | 31.96% |
| % H    | 2.89%       | 1.7%   | 2.71%  | 3.45%  | 3.41%  | 2.66%  |
| % N    | 30.43%      | 20.95% | 22.72% | 20.75% | 20.51% | 22.37% |
| Potency | H₂L        | Fe(H₂L)(OH)(H₂O) | CoH₂L(OH)(H₂O) | NiH₂L(OH)(H₂O) | Cu(H₂L)(OH)(H₂O) | ZnH₂L(OH)(H₂O) |

2.6 Isolation of bacterial and fungal pathogens

Bacterial strains were grown on its selective media as E.coli on MacConkey agar media (Oxoid; CM 0115) and EMB; Oxoid; CM 69, plates whereas, the other strains (Staphylococcus and Streptococcus) were determined on agar plates and were grown on their nutrient media. The plates were incubated at 37°C for 48 hours and the bacterial growth was observed for all of the isolates [29].

2.7 Biochemical and serological identification of bacterial pathogens

Standard Kits (Biomerieux, Marcy L’etoil, France) API had been used for both physiological and biochemical identification of each bacterial isolates of both gram positive and negative bacteria.

2.8 Minimal inhibitory concentration (MIC) measurement

For each strain, three to five isolated colonies were selected from the fresh agar plate and were transferred into a tube containing 3-4 ml of sterile broth medium. The bacterial suspension was mixed well and incubated at 35-37°C for 2-6 h. The turbidity of the bacterial suspension should be equal to or greater than the turbidity of a McFarland Standard 0.5. After that, 1mg of the tested compound (antimicrobial agent) was dissolved in 1 ml DMSO and two-fold serial dilution was done using broth medium. A fixed volume of the prepared bacterial inoculum was added to each tube and incubated for at 37°C 16-20 h. The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits visible growth of the tested isolate as observed with the unaided eye [30].

2.9 Antimicrobial assay

The antimicrobial activity of synthesized compounds was determined using agar well diffusion method⁷⁵. All the compounds were tested in vitro for their antibacterial activity against staphylococcus
aureus and Streptococcus mutans (Gram positive bacteria), Escherichia coli, Pseudomonas aeruginosa and klebsiella (Gram negative bacteria) using nutrient agar medium. Ampicillin and Gentamicin were used as standard drugs for Gram positive and Gram negative respectively. DMSO was used as solvent control. The compounds were tested at a concentration of 15 mg/ml against both bacterial and fungal strains [31].

2.10 Method of testing
The sterilized media was poured onto the sterilized Petri dishes (20-25 ml, each petri dish) and allowed to solidify at room temperature. Microbial suspension was prepared in sterilized saline equivalent to McFarland 0.5 standard solution (1.5x 10^3 CFU mL^-1) and its turbidity was adjusted to OD= 0.13 using spectrophotometer at 625 nm. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension and was flooded on the dried agar surface then allowed to dry for 15 minutes with lid in place. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer. 100 μL of the solution of the tested compound was added to each well with the help of micropipette. The plates were incubated at 37°C for 24 hrs in case of antibacterial activity. This experiment was carried out in triplicate and zones of inhibition were measured in mm [31].

3. Results and discussion

3.1. Mass spectra
The mass spectrum of the prepared of 5-(3-nitrophenyazo)-6-aminouracil ligand is shown in Scheme 2 and table 2. The mass spectral pattern of 5-(3-nitrophenyazo)-6-aminouracil showed an intense molecular ion peak at m/z 276 of relative intensity (100%), corresponding to the molecular weight of the compound with the formula C_{10}H_8N_6O_4 this intensity is a proof of its quite elevated stability. The molecular ion undergoes cleavage of C-N bond adjacent to the azo group giving two fragments with two ion peaks at m/z 154 and m/z 122 [32] corresponding to [C_6H_4 N_2O_2]^+ and [O_2N-C_6H_4]^+, respectively. The fragment [O_2N-C_6H_4]^+ loses nitric oxide molecule producing the fragment [C_6H_3O]^+ at m/z 92 and this loss is characteristic for an aromatic nitro group and this peak is usually weak but it is usually present [33] . The latter ion fragments undergoes further loss of carbon monoxide molecule producing [C_6H_4]^+ at m/z 63 [33] . The ionic fragment [C_6H_4 N_2O_2]^+ loses N_2 molecule to give the fragment [C_6H_4 N_2O_2]^+ with m/z 126 which loses a stepwise two CO molecules producing the fragments [C_6H_4 O]^+ at m/z 98 and [C_6H_2 N_2]^+ at m/z 70. The peaks observed at m/z 83 and at m/z 55 can be attributed to the loss of iso cyanic acid [HNCO] from the aromatic cycle of the uracil ring [C_6H_4 N_2O_2]^+ making a retro Diels-Alder mechanism [34] producing the ionic fragment [C_6H_2 N_2O]^+ which losses CO giving the ionic fragment [C_6H_2 N_2]^+ m/z 55 [26,35].

3.2. Infrared spectroscopy
The infrared spectral data of the5-(3-nitrophenyazo)-6-aminouracil ligand and its prepared complexes are given in figure 1 and table 3. The main purpose of the IR studies is to assign the vibrational frequencies of the fundamental groups (such as NH_2, NH, C=O, C-N and OH) of the ligand and by comparison to the infrared spectra of complexes to determine the binding sites. The infrared spectral data of the 5-(3-nitrophenyazo)-6-aminouracil ligand showed a broad band in the range 3442-3106 cm^-1 which was assigned to OH, NH and NH_2 groups [36–38], so it can be suggested that this compound exists in the enol form [36] as shown in scheme 3. The broadness of the band may be suggested that NH group is associated in intramolecular hydrogen bond with the adjacent keto...
groups. (scheme 3). The band observed at 2800-2813 cm\(^{-1}\) in both ligand and complexes is attributed to the aromatic CH stretching frequency. The symmetric deformation mode (δ) of NH\(_2\) and plane bending of N\(_1\)H and N\(_3\)H groups gives rise to its characteristic frequencies at 1511-1532 cm\(^{-1}\) for the synthesized ligand and its complexes [39–41].

**Table 2.** Relative intensity of molecular ion and major fragments of 5-(3-nitrophenylazo)-6-aminouracil ligand

| M. Wt. of fragment | Relative intensity of molecular ion >5% | Assignment |
|--------------------|----------------------------------------|------------|
| 276                | 100.00                                 | C\(_{10}\)H\(_8\)N\(_6\)O\(_4\) |
| 154                | 25.06                                  | C\(_6\)H\(_4\)N\(_2\)O\(_2\)\(^+\) |
| 126                | 11.64                                  | C\(_6\)H\(_4\)N\(_2\)O\(_2\)\(^+\) |
| 122                | 20.76                                  | C\(_8\)H\(_4\)NO\(_2\)\(^+\) |
| 98                 | 78.54                                  | C\(_6\)H\(_3\)O\(_2\)\(^+\) |
| 92                 | 19.54                                  | C\(_6\)H\(_4\)O\(^+\) |
| 83                 | 18.77                                  | C\(_3\)H\(_4\)N\(_2\)O\(^+\) |
| 76                 | 18.60                                  | C\(_2\)H\(_4\)\(^+\) |

**Table 3 (a).** Assignment of IR bands (Cm\(^{-1}\)) of 5- (3-nitrophenylazo)6- amino uracil ligand and its prepared complexes

| Ligand | Fe\(^{3+}\) | CO\(^{2-}\) | Ni\(^{2+}\) | Cu\(^{2+}\) | Zn\(^{2+}\) | Assignment |
|--------|------------|------------|------------|------------|------------|------------|
| -------|------------|------------|------------|------------|------------|------------|
| 3442(mb)| 3594(w)    | 3686-3000  | 3689(w)    | 3610(w)    | 3527(w)    | ¥NH2, ¥OH of H2O and OH |
| 3000(mb)| 3342-3000  | 3664(mb)   | 3306(m)    | 3282       | 3289       | ¥NH amide |
| 3258(mb)| 3175(mb)   | 3198       | 3123(m)    | 3098(m)    | 3007(w)    | ¥CH aromatic |
| 3107(m) | 3085(mb)   | 2977(w)    | 2980(s)    | 2977(m)    | 3007(w)    | Strong H bond complex |
| 2806(w) | 2806(w)    | 2886(w)    | 2903(m)    | 2800(w)    | 2813(w)    | ¥C=O amide |
| 1744(s) | 1737(w)    | 1725(m)    | 1702(w)    | 1727(w)    | 1737(m)    | ¥C=O amide |
Table 3 (b). Assignment of IR bands (Cm$^{-1}$) of 5- (3-nitro phenyl azo)6- amino uracil ligand and its prepared complexes

| 1633(s) | 1625(m) | 1627(m) | 1605(m) | 1621(m) | 1600(m) | $\gamma$ C=O + $\gamma$ C=C |
|---------|---------|---------|---------|---------|---------|---------------------------|
| 1532(m) | 1523(m) | 1520(s) | 1517(m) | 1511(s) | 1518(m) | $\delta$ NH$_2$ + $\delta$ N$_2$H |
|         |         |         |         |         |         | a symmetricNO$_2$ |
| 1449(w) | 1437 (w) | 1396(s) | 1389(m) | 1409(m) | -------- | N=N, $\delta$N$_3$H, |
| 1350(w) | 1346(m) | 1342(s) | 1346(m) | 1347(m) | 1344(m) | symmetricNO$_2$ |
| 1257(m) | 1255(W), | 1247(w) | 1238(m) | 1216(m) | 1264(w) | $\gamma$ ring stretching C-N |
| 1093(m) | 1090(w) | 1082(m) | 1065(m) | 1072(m) | 1081(w) | P NH$_2$C-O,C-N |
| 892(w)  | 888(w)  | 876(s)  | 886(m)  | 888(m)  | 889(w)  | $\gamma$ ring stretching |
| 811 Bending | 807(m) | 801(m) | 808(w) | 814(w) | 803(s) | $\gamma$ ring stretching due to 1,3 di substitution |
| 740     | 733(w)  | 730(m)  | 736(w)  | 740(m)  | 735(w)  | +WNH$_2$ |
| 675     | 666(w)  | 665(s)  | 670(w)  | 681(m)  | 665(m)  |             |
| 631(m)  | ------  | ------  | ------  | ------  | ------  | $\gamma$ N-C=O |
| -------- | 514(m)  | 502(m)  | 586(w)  | 576(s)  | 520(m)  | T NH$_2$, Metal-Nitrogen |
| -------- | 425(w)  | 426(m)  | 495(m)  | 434(m)  | 421(w),45 | Metal- Oxygen |

The two sharp strong intense peaks observed at 1744 and 1633 cm$^{-1}$ are attributed to the carbonyl groups at C$_2$=O and C$_4$=O positions of the uracil ring respectively [42]. The bending deformation vibration band of the C-NH$_2$ is observed at 1532 cm$^{-1}$ and the stretching vibration band of the azo group is assigned to be at 1449 cm$^{-1}$ [43–47]. The band observed at 1257 cm$^{-1}$ is due to stretching vibration of C-N in the ligand. The presence of 3 bands at 811,740 and 675 cm$^{-1}$ in the ligand spectrum are due to C-NO$_2$ at the meta position of benzene ring. The infrared spectra of the complexes showed a strong broad band extended from 3689 - 3000 cm$^{-1}$ corresponding to the coordinated H$_2$O involved in the complexation to satisfy the coordination number and OH group involved in the complexes to
satisfy the oxidation number of metal cation. This broad band involves also the stretching bands of NH and NH₂ and phenolic CH of uracil ring.

**Figure 1.** Mass spectrum of 5-(3-nitro-phenylazo)-6-aminouracil

**Scheme 2.** Fragmentation pattern of 5-(3-nitro phenyl azo) 6-aminouracil
Scheme 3. Tautomer forms of 5-(m-nitro phenyl azo) 6-aminouracil ligand

The shift of bending vibration band of both NH$_2$ from 1532 to 1511 cm$^{-1}$ and C-NH$_2$ deformation from 1264 to 1216 cm$^{-1}$ confirm that the amino NH$_2$ group is participated in chelation. Also, the shift in stretching vibration band of the azo group from 1449 to 1389 cm$^{-1}$ in all complexes (except zinc complex that disappeared in it) confirm that chelation occurred through the amino NH$_2$ group and the azo N=N group. Two new bands within the range 576-502 cm$^{-1}$ and 495-421 cm$^{-1}$ observed in the spectra of the complexes and not observed in the spectrum of are ligand were due to M-O and M-N. The IR spectra revealed that the ligand behaves as negative bidentate ligand through deprotonated of NH$_2$ group of uracil ring and N=N of azo group.

Scheme 4. Complexation pattern between metal cation and 5-(m-nitro phenyl azo) 6-aminouracil forming hydrogen bond

3.3. $^1$HNMR spectral study

The $^1$HNMR spectral data - listed in table 4 and represented by figure 3 show that the ligand exhibits a doublet band at 2.50 ppm assigned to the CH$_3$ protons of the solvent (DMSO-d$_6$) and a multiple band within the range 7.10 – 7.71 ppm representing the 4 CH protons of the phenyl ring of the 5-(3- nitro phenyl azo)-6-aminouracil ligand. The two NH protons in positions 1 and 3 in the uracil ring exhibited two singlet peaks at 10.61-11.01 ppm, respectively [47,48]. The two NH$_2$ protons in the 6 position of the uracil ring were observed at 3.17 ppm which is shifted to 3.40 ppm in the complexes due to chelation as shown in figure 5. To recognizing the exchangeable protons in the compounds, one drop of D$_2$O is added to the sample resulting in the disappearance of the two NH signals indicating that the protons are linked to the two oxygen atoms of the two adjacent carbonyl oxygen producing the enolic form illustrated by scheme 5 and figure 4.
The $^1$HNMR spectra of the complexes figure 3 showed an additional singlet broad band at 3.5 ppm corresponding to the coordinated water and the OH satisfy the oxidation number on the metal ion as shown in figure 5.

![Infrared spectra of the complexes](image)

**Figure 2.** Infrared spectra of the 5- (3-nitro phenyl azo)6- amino uracil ligand and its prepared complexes; A---IR of ligand B----- IR of Fe-complex C-----IR of Co-complex D---- IR of Ni-complex E----- IR of Cu-complex F----- IR of Zn- complex.

The $^1$HNMR spectra of the complexes figure 3 showed an additional singlet broad band at 3.5 ppm corresponding to the coordinated water and the OH satisfy the oxidation number on the metal ion.

![Scheme 5](image)

**Scheme 5.** Keto-enol equilibrium for 5-(3- nitro phenyl azo)-6-aminouracil ligand.
Figure 3. HNMR of 5-(3-nitro phenyl azo) 6-aminouracil

Figure 4. Duet rated NMR of 5-(m-nitro phenyl azo) 6-aminouracil
### Table 4. Protocol of proton NMR spectra data of 5-(3-nitro phenylazo)-6-aminouracil ligand complex and ligand

| Node | Theoretical (ppm) | Shift found (ppm) | Comment |
|------|------------------|------------------|---------|
| NH₂  | 8.46             | 3.17             | Amine+ H bond |
| N₃H  | 10.00            | 11.01            | Imide |
| N₁H  | 6.00             | 10.61            | Urea |
| CH   | 7.26             | 7.10–7.71        | 4H Benzene |
| H₂O, OH | 2               | 3.5              | Coordinated H₂O, OH |

#### Figure 5. HNMR of Ni-5-(m-nitro phenyl azo) 6-aminouracil as represented element

3.4. **Thermal analysis**

The TGA and DTA of complexes were carried out in order to (i) get information about thermal stability of these new complexes, (ii) decide whether the water molecules are inside or outside the inner coordinated sphere of the central metal ion because it is impossible to differentiate between hydrated and coordinated water molecules by using IR spectral data, and (iii) suggest a general scheme for thermal decomposition of these complexes. The coordinated water molecules are more strongly bonded to the metal ion than water molecules of hydration, and hence are eliminated at higher temperature than those of water of hydration.
Scheme 6. Thermal degradation of 5-(3-Nitro phenylazo)-6-aminouracil

Thermal analyses data (TGA and DTA) listed in table 5 showed that 5-(3-Nitro phenylazo)-6-aminouracil undergoes an exothermic decomposition by losing the NH$_2$ at 147 °C. Further heating leads to the release of uracil ring at 344 °C. The azo group is released at 438 °C. The phenyl ring is released at 581 °C [27] and finally the NO$_2$ is released at 823 °C.

The Fe(III) complex decomposes in three phases. The first phase corresponds to the loss of a molecule of H$_2$O between 26–127°C with mass losses of (obs. = 4.41%, calc. = 4.49%) (52). The second phase is from 210 to 450°C and is attributed to the loss of the organic moiety C$_{20}$H$_{18}$NS with mass losses of (obs. = 44.37%, calc. = 44.07%). The final phase shows the loss of the organic moiety, C$_8$H$_{10}$OS, at 450–700°C with mass losses of (obs. = 22.53%, calc. = 22.33%) leaving Fe$_2$O$_3$ as the final product, and the fragment C$_6$N is lost as 8CO$_2$ and 0.5N$_2$.

The decomposition of the Co(II) complex also occurred in four steps. The first step is due to the loss of a molecule of water and 2H at 41–164°C, with mass losses of (obs. = 5.72%, calc. = 5.42%). The successive decomposition occurs within a temperature range of 164–326°C and is attributed to the loss of the organic moiety CHN$_2$ with mass losses of (obs. = 11.12%, calc. = 10.84%). The third step is due to the loss of CH$_4$N$_2$O at 326-408°C, with mass losses of (obs. = 16.65%, calc. = 16.26%). The last step involves the loss of the organic moiety, C$_6$NO$_2$, at 408-812°C with mass losses of (obs. = 30.96%, calc. = 31.98%). The final product is CoO, and the C$_5$ fragment is lost as CO and CN.

The TGA curve of the Ni(II) complex reveals a two-step decomposition. The first is the loss of two molecules of water at 50–191°C, with mass losses of (obs. = 9.11%, calc. = 8.89%). The second step ranges from 288 to 484°C and is assigned to the loss of the organic moiety, C$_{10}$H$_8$N$_6$O$_3$, with mass losses (obs. = 72.44%, calc. = 72.64%). The overall weight loss (81.55%) calc and (81.57%) found leaving NiO as residue.
Table 5 (a). Thermal analyses data (TG-DTA) of 5-(3-nitro phenylazo)-6-aminouracil and its complexes

| Compound       | Temp. range [°C] | Tm [°C] | Peak type | Calc. weight loss [%] | found weight loss [%] | Losing fragment |
|----------------|------------------|---------|-----------|-----------------------|-----------------------|-----------------|
| Ligand         | 103-196          | 345     | Exothermic| 5.80                  | 6.01                  | NH$_2$          |
|                | 280-400          |         | Exothermic| 39.85                 | 39.80                 | C$_6$H$_2$N$_2$O$_2$ |
|                | 403-493          |         | Exothermic| 10.15                 | 10.28                 | N=N             |
|                | 497-697          |         | Exothermic| 27.17                 | 27.23                 | C$_6$H$_5$      |
|                | 800-936          |         | Exothermic| 16.67                 | 16.14                 | NO$_2$          |
| [Fe(H$_2$L)(OH)$_2$]$_2$(H$_2$O)$_2$ | 26-127          | 308     | Endothermic| 4.49                  | 4.41                  | H$_2$O          |
|                | 127-316          |         | Exothermic| 18.21                 | 18.43                 | C$_3$H$_5$N$_2$O |
|                | 316-451          |         | Exothermic| 19.20                 | 19.11                 | C$_3$H$_3$N      |
|                | 451-564          |         | Endothermic| 9.48                  | 9.63                  | C$_2$N          |
|                | 564-1096         |         | Endothermic| 19.70                 | 20.06                 | H$_2$N$_2$O$_3$  |

\[ \text{Residue} + \text{CO} \]
Table 5 (b). Thermal analyses data (TG-DTA) of 5-(3-nitro phenylazo)-6-aminouracil and its complexes

| Complex                      | Temperature (°C) | Type      | Endothermic | Exothermic | Dehydration of | Final  |
|------------------------------|------------------|-----------|-------------|------------|----------------|--------|
| [CoH₂L(OH)(H₂O)]            | 41-164           | 351       | Endothermic | 5.42       | CH₂N₂       | 10.84  |
|                              | 164-326          |           |             | 10.84      |               | 11.12  |
|                              | 326-408          |           |             | 16.26      | CH₄N₂O      | 16.55  |
|                              | 408-812          |           |             | 31.98      | C₆HNO₂       | 30.96  |
|                              |                  |           | Exothermic  | 34.94      | Residue       | 34.57  |
|                              |                  |           |             |            | [Co O+]CO+CN |        |
| [NiH₂L(OH)(H₂O)2H₂O]        | 46-191           | 416       | Weak endothermic | 8.89       | 2 H₂O         | 9.11   |
|                              | 288-484          |           | Strong endothermic | 72.64      | C₄H₆NO₂     | 72.42  |
|                              |                  |           |             | 18.45      | +C₄H₃N₂O₂+H₂O |        |
|                              |                  |           |             |            | Residue       | 18.47  |
|                              |                  |           |             |            | NiO           |        |

Cu(II) complex decomposes in four steps. The first step is attributed to the loss of the two molecules of water, with mass losses of (obs. = 9.35%, calc. = 8.79%) at 13–201°C. The second step ranges from 201 to 300°C and is attributed to the loss of the fragment H₂O and 2H, with mass losses of (obs. = 5.00%, calc. = 4.88%). The third step ranges from 300 to 617°C and is attributed to the loss of the organic moiety C₄H₂N₃O₂, with mass losses of (obs. = 31.88%, calc. = 30.27%). The final step is from 617 to 1101°C corresponding to the loss of the CHN₂, with mass losses of (obs. = 10.78%, calc. = 10.49%). The remaining residue is mixture of CuO and C₄H₃NO₂.
Table 5 (c). Thermal analyses data (TG-DTA) of 5-(3-nitro phenylazo)-6-aminouracil and its complexes

| Complex                        | TG Temperature | DTA Type     | Endothermic | Exothermic | Mass Loss (%) | Final Product       |
|--------------------------------|----------------|--------------|-------------|------------|---------------|---------------------|
| [Cu(H₂L)(OH)(H₂O)]₂H₂O        | 13-201         | Endothermic  | 8.79        | 9.35       | 2H₂O          | H₂O+2H              |
|                                | 201-300        | Exothermic   | 4.88        | 5.00       | H₂O           | C₄H₂N₃O₂            |
|                                | 300-617        | Exothermic   | 30.27       | 31.88      | CHN₂          | Residue             |
|                                | 617-1101       | Exothermic   | 10.49       | 10.72      | 43.10         | Residue             |
|                                |                |              |             |            | 43.32         | Residue             |
| [ZnH₂L(OH)(H₂O)]               | 34-168         | Endothermic  | 4.79        | 3.71       | H₂O           | C₆H₄N₂O₂            |
|                                | 168-340        | Exothermic   | 7.99        | 7.92       | N₂+2H        | ZnO                 |
|                                | 340-550        | Endothermic  | 29.30       | 29.44      | C₃H₆N₂O₂      | Residue             |
|                                | 550-1101       | Endothermic  | 36.22       | 36.20      | C₃H₆N₂O₂      | ZnO                 |

The Zn(II) complex decomposes in four steps. Step one is between 34–168°C, which indicates the loss of H₂O, with mass losses of (obs. = 3.71%, calc. = 4.79%). The second step involves the loss of azo group and 2H from 168 to 340°C, with mass losses of (obs. = 7.92%, calc. = 7.99%). The third step involves the loss of the organic moiety C₄H₂N₂O₂, from 340 to 550°C, with mass losses of (obs. = 29.44%, calc. = 29.30%). The final step is attributed to the loss of the organic moiety, C₃H₆N₂O₂, at 550–1101°C, with mass losses of (obs. = 36.20%, calc. = 36.22%), leaving behind the ZnO residue and the fragment C₃ is lost as 3CO₂.
Scheme 7. Thermal degradation of Fe (III) complex.

Scheme 8. Thermal degradation of Co(II) complex.
Scheme 9. Thermal degradation of Ni (II) complex.

Scheme 10. Thermal degradation of Cu (II) complex.
Scheme 11. Thermal degradation of Zn (II) complex.

In all cases with the exceptions of the ligand and the Cu(II) complex, the decomposition pattern showed the loss of carbon fragments which got oxidized to CO$_2$, and hydrogen or nitrogen which were lost as gases. Thus, the decomposition pattern corroborates the proposed formulation of the complex.

3.5. Magnetic moment measurements

On the basis of the magnetic moment measurements lifted in Table 6 it is found that all the complexes under study are paramagnetic except for the diamagnetic zinc complex that shows square planar geometry because of the exact value of magnetic moment depends on the temperature of measurement and the magnitude of the spin-orbit coupling, all measurements were carried out at room temperature. In the present investigation, Fe$^{3+}$ complex has magnetic moment of about 2.3 [B.M] indicating low-spin octahedral state which corresponds to the presence of one unpaired electron. Co$^{2+}$ complexe has a magnetic moment value of 4.86BM at room temperature, suggesting tetrahedral geometry for this complex which is confirmed by the presence of 3 unpaired electrons. The observed magnetic moment value for Ni$^{2+}$is 3.75 (BM) which falls in the range of tetrahedral and square planar structure for this complex but the presence of 2 unpaired electrons excludes Square planar geometry and confirms tetrahedral structure for the complex. The Cu$^{2+}$ complex, under investigation showed magnetic moment of 2.4[BM] which suggested tetrahedral structure that supported by thermal analysis of this complex. The relatively high magnetic moment value can be attributed to ferromagnetism on the complex [49–54].
Table 6. The magnetic susceptibility measurements, diamagnetic corrections and the effective magnetic moments of Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ complexes:

| Complex | $X_M$ $10^{-6}$ Cgs unite | $X_D$ $10^{-6}$ Cgs unite | $X_{M_c}$ $10^{-6}$ Cgs unite | $M_{eff}$ Theo | $M_{eff}$ | Number of unpaired e | Geometry |
|---------|-----------------|-----------------|-----------------|--------|--------|-----------------|----------|
| [Fe(H$_2$L)(OH)$_2$(H$_2$O)$_2$] | 5.5468 | 2.2334 | 12.8 | 2.2106 | 2.30 | 2-2.5 | 1 Octahedral(low spin) |
| [Co(H$_2$L)(OH)(H$_2$O)] | 2.5032 | 9.2350 | 12.8$^*$ | 9.2222 | 4.688 | 3.87 | 3 Tetrahedral |
| [Ni(H$_2$L)(OH)(H$_2$O)]2H$_2$O | 1.4602 | 5.9092 | 12.8$^*$ | 5.8964 | 3.75 | 2.83 | 2 Tetrahedral |
| [Cu(H$_2$L)(OH)(H$_2$O)]2H$_2$O | 5.95 | 2.4375 | 12.8$^*$ | 2.4247 | 2.40 | 1.7-2.2 | 1 Tetrahedral |
| [Zn(H$_2$L)(OH)(H$_2$O)] | 4.542 | 1.705 | 13 | 1.692 | 0 | 1.7 | 0 Square planar |

$X_M$ = Molar susceptibility
$X_D$ = Diamagnetic susceptibility
$X_{M_c}$ = Corrected Molar susceptibility
$M_{eff}$ = Effective Magnetic moment

Scheme 12. Geometry of Fe-5-(m-nitro phenyl azo) 6-aminouracil complex

Scheme 13. Geometry of Co-5-(m-nitro phenyl azo) 6-aminouracil complex

Scheme 14. Geometry of Ni-5-(m-nitro phenyl azo) 6-aminouracil complex
Scheme 15. Geometry of Cu-5-(m-nitro phenyl azo) 6-aminouracil complex

Scheme 16. Geometry of Zn-5-(m-nitro phenyl azo) 6-aminouracil complex

3.6. The antibacterial assay

Table 7 shows the diameters of the inhibition zones of the different bacterial strains against gram positive and gram negative bacteria. Also Figure 6 shows the inhibition zone (mm) of the cobalt and Zinc complexes. Besides the anti-microbial activity increased against gram positive indicated with increased zone of inhibition than negative one but the showed also activity against gram negative with anti-fungal activity.

Table 7. The diameters of the zones against different bacterial strains

| Microorganism                  | Ligand          | Ni Complex | Fe Complex | Cu Complex | Zn Complex | Co Complex | Standard antibiotic |
|--------------------------------|-----------------|------------|------------|------------|------------|------------|---------------------|
| **Gram negative bacteria**     | Gentamicin      |            |            |            |            |            |                     |
| Escherichia coli               | NA              | 22.6±0.5   | NA         | 11±0.5     | 27         | 25         | 34±0.1              |
| Klebsiella pneumonia           | NA              | NA         | NA         | NA         | 27         |            | 45±0.5              |
| Pseudomonas aeruginosa         | NA              | NA         | NA         | NA         | -          | -          | 35±0.4              |
| **Gram positive bacteria**     | Ampicillin      |            |            |            |            |            |                     |
| Staphylococcus aureus          | NA              | NA         | NA         | NA         | NA         | NA         | 33±0.2              |
| Streptococcus mutans.          | NA              | 9.6±0.5    | NA         | 29±1       | 27.6±0.5   |            | 22±0.1              |
| **Fungi**                      | Nystatin        |            |            |            |            |            |                     |
| candida albicans               | NA              | 15.3±0.5   | NA         | NA         | 17.3±0.5   | 28±0.2     |                     |
The current research studied the antibacterial activity of different complexes on different bacterial strains besides their antifungal activities. Especially both cobalt and zinc nanocomposites showed good antibacterial activity against gram-positive and gram-negative bacteria. Regarding the cobalt antibacterial activity that’s might be attributed to the cobalt nanomaterial have the ability for cell membrane attachment and disruption so increase the bacterial permeability with bactericidal activity. In addition cobalt in nano size had the ability for interacting with the sulfur- and phosphorus-containing compounds in the bacterial cell lead to lose the bacterial activity. Besides cobalt in nano form can interact with the DNA of the bacterial cells causes’ damage in the DNA and stop its replications besides bacterial cell death. Cobalt ion itself when released from the nano-composites causes severe antibacterial activity [55].

Figure 6. Shows the Zone of inhibition (mm) of both cobalt and zinc complexes against streptococcal infection

Zinc complex also showed good antibacterial activity in this study which might be attributed to the presence of the water molecules which responsible for generating free radicals like hydroxyl or oxygen species that responsible for the oxidative stress and antimicrobial activity.

4. Conclusion
5-(3-nitrophenyllazo)-6-minouracil behaves as monobasic bi dentate ligand that bonds to the metal ion through both NH₂ group and N=N groups forming six membered ring. These complexes have high thermal stability and are biologically active towards bacteria gram (-ve), gram (+ ve) and fungi.

References
[1] Seferoglu Z and Ertan N 2007 Synthesis and spectral properties of new hetarylazo indole dyes Russ. J. Org. Chem. 43 1035–41
[2] Seferoglu Z and Ertan N 2007 Synthesis of some novel bis(hetaryl)azo disperse dyes and investigation of their absorption spectra Heteroat. Chem. 18 622–30
[3] Y.H.MARIUM R B M and 1973 Metal Ions in Biological Systems: Volume 8
[4] Sujamol M S, Athira C J, Sindhu Y and Mohanan K 2010 Synthesis, spectroscopic characterization, electrochemical behaviour and thermal decomposition studies of some transition metal complexes with an azo derivative Spectrochim Acta A Mol Biomol Spectrosc 75 106–12
[5] Diab M A, El-Bindary A A, El-Sonbati A Z and Salem O L 2012 Supramolecular structure and substituents effect on the spectral studies of dioxygenorium(VI) azodyes complexes J. Mol. Struct. 1007 11–9

[6] Hassanzadeh A, Zeini-Isfahani A, Habibi M H, Heravi M R A P and Abdollahi-Alibeik M 2006 1H, 13C, NH, HH, CH COSY, HH NOESY NMR and UV–vis studies of Solophenyl red 3BL dye azo-hydrazone tautomerism in various solvents Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 63 247–54

[7] Pirillo S, Einschlag F S G, Ferreira M L and Rueda E H 2010 Erichrome Blue Black R and Fluorescein degradation by hydrogen peroxide oxidation with horseradish peroxidase and hematin as biocatalysts J. Mol. Catal. B Enzym. 66 63–71

[8] Pirillo S, Rueda E H and Ferreira M L 2012 Supported biocatalysts for Alizarin and Erichrome Blue Black R degradation using hydrogen peroxide Chem. Eng. J. 204–205 65–71

[9] Rageh N M 2004 Tautomeric structures, electronic spectra, acid–base properties of some 7-aryl-2,5-diamino-3(4-hydroxyphenyazo)pyrazolo[1,5-a]pyrimidine-6-carbonitriles, and effect of their copper(II) complex solutions on some bacteria and fungi Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 60 1917–24

[10] Li X, Wu Y, Gu D and Gan F 2010 Spectral, thermal and optical properties of metal(II)–azo complexes for optical recording media Dye. Pigment. 86 182–9

[11] Masoud M S, Ibrahim A A, Khalil E A and El-Marghany A 2007 Spectral properties of some metal complexes derived from uracil–thiourea and citrazinic acid compounds Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 67 662–8

[12] Bayrak C 2012 Vibrational spectroscopic study of 6-aminouracil tetracyanonickelate complexes Hacettepe J. Biol. Chem. 40 419–26

[13] Edupuganti R, Wang Q, Tavares C D J, Chitjian C A, Bachman J L, Ren P, Anslyn E V and Dalby K N 2014 Synthesis and biological evaluation of pyrido[2,3-d]pyrimidine-2,4-dione derivatives as eEF-2K inhibitors Bioorg. Med. Chem. 22 4910–6

[14] Pałasz A and Cież D 2015 In search of uracil derivatives as bioactive agents. Uracils and fused uracils: Synthesis, biological activity and applications Eur. J. Med. Chem. 97 582–611

[15] El-Barbary A A, Hafiz Y A and Abdel-Wahed M S 2011 Synthesis, characterization, and biological activity of some aza-uracil derivatives J. Heterocycl. Chem. 48 639–44

[16] Holm R H, Kennespohl P and Solomon E I 1996 Structural and Functional Aspects of Metal Sites in Biology Chem. Rev. 96 2239–314

[17] Akbar Ali M and Livingstone S E 1974 Metal complexes of sulphur-nitrogen chelating agents Coord. Chem. Rev. 13 101–32

[18] Singleton P 1999 Bacteria in Biology, Biotechnology and Medicine vol 5th ed.

[19] Teniaillon, Olivier; Skurnik, David; Picard, Bertrand; Denamur E 2015 The population genetics of commensal Escherichia coli Nat. Rev. Microbiol. 8 207–17

[20] Masalha M, Borovok I, Schreiber R, Aharonovitz Y and Cohen G 2001 Analysis of transcription of the Staphylococcus aureus aerobic class Iib and anaerobic class III ribonucleotide reductase genes in response to oxygen J. Bacteriol. 183 7260–72

[21] Ghosh P, Mukhopadhyay T K and Sarkar A R 1984 Interaction of divalent metal ions with uracil III. Complexes of MnII, FeII, CoII, NiII and CuII with uracil acting as bidentate ligand Trans. Met. Chem. 9 46–8

[22] Ali O Y and Fridgen T D 2011 Structures of electrosprayed Pb(Uracil-H)+ complexes by infrared multiple photon dissociation spectroscopy Int. J. Mass Spectrom. 308 167–74

[23] Narang K K, Singh V P and Bhattacharya D 1997 Synthesis, characterization and antitumour activity of uracil and uracil–histidine complexes with metal(III) ions Transl. Met. Chem. 22 333–7

[24] Singh V P, Singh A, Narang K K and Bhattacharya D 2004 5-Bromouracil and 5-bromouracil–histidine complexes with metal(III) ions and their antitumour activity Transl. Met. Chem. 29 107–11

[25] Singh V P, Singh S, Narang K K and Bhattacharya D 2009 Aluminium(III), chromium(III) and iron(III) complexes with 5-iodouracil and 5-iodouracil-histidine and their antitumour
activity J. Enzyme Inhib. Med. Chem. 24 105–10

[26] Moanta A, Ionescu C, Tutunaru B and Dumitru M 2010 Thermal and electron impact decomposition of 4-hydroxy-4’-cyano-azobenzene Rev. Chim. 61

[27] Schwarzenbach G 1957 Complexometric titrations. vol 89 (Methuen Co., London)

[28] Vogel A I 1961 A text book of Quantitative Inorganic Analysis, including elementary instrumental analysis

[29] Collee JG, Miles RS W B 2006 Tests for identification of bacteria. In Collee JG, Fraser AG, Marmion BP, Simmons A. eds. Mackie and McCartney’s Practical medical microbiology, 14th ed (Churchill Livingstone)

[30] Wiegand I, Hilpert K and Hancock R E W 2008 Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances Nat. Protoc. 3 163–75

[31] C.Scott A 1989 Laboratory control of antimicrobial therapy. In: Collee JG et al. eds. Practical Medical Microbiology, 13th Edition (Edinburgh: Churchill Livingstone)

[32] Bernier J-L and Hénichart J-P 2018 Electron impact fragmentation of 6-aminouracils Biomed. Mass Spectrom. 10 626–8

[33] Moanta A 2012 Characteristic Fragmentation Patterns of Some 4- (phenylazo ) phenols Obtained by Electron Impact Mass Spectrometry Rev. Chim. 63 7–9

[34] Sadr-Arani L, Mignon P, Chermette H, Abdoul-Carime H, Farizon B and Farizon M 2015 Fragmentation mechanisms of cytosine, adenine and guanine ionized bases Phys. Chem. Chem. Phys. 17 11813–26

[35] Issa Y M, Hassan B H and Abdelaal H E 2009 1H NMR, 13C NMR and mass spectral studies of some Schiff bases derived from 3-aminoo-1,2,4-triazole Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 74

[36] Curtis N F 1972 Some metal-ion complexes with ligands formed by reaction of amines with aliphatic carbonyl compounds. Part I. Nickel(II) and copper(II) complexes formed by the diaminothioacetone-acetone reaction J. Chem. Soc. Dalt. Trans. 1357–61

[37] Ruiz-Sánchez J, Colacio-Rodriguez E, Salas-Peregrin J M and Romero-Molina M A 1986 Thermal decomposition of 6-aminoo-1,3-dimethyl-5-phenylazouracil complexes of Co(II), Ni(II), Cu(II) and Ag(I) J. Anal. Appl. Pyrolysis 9 159–70

[38] Masoud M S, Abou El-Enein S A, Ayad M E and Goher A S 2004 Spectral and magnetic properties of phenylazo-6-aminouracil complexes Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 60 77–87

[39] Yadav R A, Yadav P N S and Yadav J S 1988 Vibrational studies of biomolecules—II. 2-thiocytosine Spectrochim. Acta Part A Mol. Spectrosc. 44 1201–6

[40] Durig J R 1993 Vibration Spectra and Structure vol 20 (Elsevier science publishers)

[41] Colombo L and Kirin D 1986 Raman spectrum of 1-methyl-uracil single crystal. Interpretation of internal and external spectra Spectrochim. Acta Part A Mol. Spectrosc. 42 557–65

[42] Debnath D, Roy S, Li B-H, Lin C-H and Misra T K 2015 Synthesis, structure and study of azo-hydrazone tautomeric equilibriums of 1,3-dimethyl-5-(arylazo)-6-aminouracil derivatives Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 140 185–97

[43] Krause R A and Krause K 1980 Chemistry of bipyridyl-like ligands. Isomeric complexes of ruthenium(II) with 2-(phenylazo)pyridine Inorg. Chem. 19 2600–3

[44] Masoud M S and Refaat L S 1982 Synthesis and characterization of some nickel(II) Schiff bases complexes Transist. Met. Chem. 7 315–8

[45] Ahmed F, Dewani R, Pervez M K, Mahboob S J and Soomro S A 2016 Non-destructive FT-IR analysis of monoazo dyes Bulg. Chem. Commun. 48 71–7

[46] Larkin P J 2011 Infrared and Raman spectroscopy : principles and spectral interpretation

[47] Seferoğlu Z and Ertan N 2008 Synthesis, characterization and spectroscopic properties of some new phenylazo-6-aminouracil Cent. Eur. J. Chem. 6 81–8

[48] Seferoğlu Z 2009 A study on tautomeric equilibria of new hetarylazo-6-aminouracils Arkivoc 2009 42–57

[49] Lewis J and Wilkins R G 1960 Modern Coordination Chemistry: Principles and Methods
[50] Angelici R J 1977 *Synthesis and Technique in Inorganic Chemistry (Saunders golden sunburst series)* (W.B. Saunders Company)

[51] Sharpe C E H and A G 2008 *Inorganic Chemistry* (Pearson Education Ltd, Harlow)

[52] Evans D F 1959 400. The determination of the paramagnetic susceptibility of substances in solution by nuclear magnetic resonance *J. Chem. Soc.* 2003

[53] Szafran Z, M. Pike R and M. Singh M 1991 *Microscale Inorganic Chemistry- A comprehensive laboratory experience*

[54] Mcmills L, Nyasulu F and Barlag R 2014 Magnetic Susceptibility of Coordination Compounds in the General Chemistry Laboratory *J. Lab. Chem. Educ.* 2 11–4

[55] El-Shahawy A A G, Abo El-Ela F I, Mohamed N A, Eldine Z E and El Rouby W M A 2018 Synthesis and evaluation of layered double hydroxide/doxycycline and cobalt ferrite/chitosan nanohybrid efficacy on gram positive and gram negative bacteria *Mater. Sci. Eng. C* 91 361–71