NEW COMPLEXES OF UREA WITH Hg(II) AND Ni(II) METAL IONS

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The complexation of urea (U) with mercury(II) and nickel(II) ions at room temperatures and 60 °C has been studied by many methods namely elemental analysis, magnetic susceptibility, conductivity measurements, infrared and thermal analysis (TG/DTG), scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX), and x-ray powder diffraction (XRD) method. The physical and spectral data were well explained in terms of the formation of $HgCl_2.4U.2H_2O$ (**1**) and $Ni(NO_3)_2.2U.2H_2O$ (**2**) at room temperature, on one hand, and $HgCl_2.2U.2H_2O$ (**3**) and on the other hand $Ni(NO_3)_2.2U.H_2O$ (**4**) at 60 °C. On the basis of the infrared spectral data and the values of stretching vibrational bands of both –C=O and –NH$_2$ groups, the complexation of metal ions toward urea was distinguished. The enhancement of the microbial treatments against bacteria (*Escherichia Coli*, *Staphylococcus Aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus Flavus* and *Candida Albicans*) was assessed and a remarkable efficiency was recorded for complexes (**1**) and (**2**).

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Introduction

Urea, CH_4N_2O, a white solid which melts at 132°C, soluble in water and ethanol, but insoluble in ether was first prepared by Wöhler [1] by evaporating a solution containing a mixture of potassium isocyanate and ammonium sulphate. Ammonium isocyanate, which is formed first, undergoes molecular rearrangement to give urea, as shown by the following reaction;

$$NH_4NCO \leftrightarrow H_2NCONH_2 \qquad (1)$$
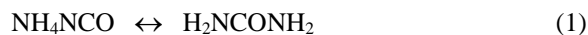
Urea can be prepared in the laboratory by the reaction of ammonia with carbonyl chloride, alkyl carbonates, chloroformates or urethans. Industrially urea is used[2-4] for preparing various formaldehyde-urea resins (plastics),[5] barbiturates[6], and fertilizers.[7-10] Urea is also extensively used in the paper industry to soften cellulose and has been used to promote healing in infected wounds and also in many other applications in the field of medicine.[11-13]. Recently, urea is used for the manufacture of hydrazine in which it is treated with alkaline sodium hypochlorite[6] and with reactive dyeing.[14]

Complexes of urea with some metal ions such as $[Zn(urea)_6]SO_4.H_2O$ and $[Zn((urea)_4](NO_3)_2.2H_2O$ are used as fertilizers[15-18]. Crystal structure studies of urea showed that in solid urea, both of the nitrogen atoms are identical.[6] Urea usually coordinates as a monodentate ligand through the oxygen atom, forming a C=O· · ·M bond. The rare N,O-bidentate coordination mode has been observed in a very limited number of cases.[19-22]

Raman and infrared spectra of urea have been studied by several scientists.[23-33]

Urea possesses two types of potential donor atoms, the carbonyl oxygen and amide nitrogens. Penland et al.[34] studied the infrared spectra of urea complexes to determine whether coordination occurs through oxygen or nitrogen atoms. If coordination occurs through nitrogen CO stretching frequency is expected to increase with a decrease of CN stretching frequency, the NH stretching frequency in this case may fall to the same range as those of the amido complexes.

On the other hand if coordination occurs through oxygen CO stretching frequency decreases but with no appreciable change in NH stretching frequency. Since the vibrational spectrum of urea itself has been analysed,[32] band shifts caused by coordination can easily be checked, for example, the effect of the coordination on the spectra of the complexes of urea with Pt(II) and Cr(III) in which the coordination occurs through nitrogen and oxygen atoms, respectively.[34] The mode of coordination of urea with metal ions seems to be dependent upon the type and nature of metal ions. Pd(II) coordinates to the nitrogen, whereas Fe(III), Zn(II), and Cu(II) coordinate to the oxygen of urea.[34]

In urea-metal complexes, if a nitrogen-to-metal bond is present, the vibrational spectrum of this complex differs significantly from that of the free urea molecule. The N-H stretching frequencies would be shifted to lower values, and the C=O bond stretching vibration, (ν(C=O)) would be shifted to higher frequency at about 1700cm^{-1}.[35]

Recently, urea represents not only an important molecule in biology[36] but also an important raw material in chemical industry.[37]

Experimental

Materials

HgCl$_2$, Ni(NO$_3$)$_2$.6H$_2$O and methanol were obtained from Aldrich Company. Urea was received from Fluka chemical company. All chemicals used in this study were of analytical grade and they were used as such without further purification.

Synthesis of Hg(II)) and Ni(II) urea complexes

Synthesis of urea complexes at room temperature

The complexes with composition of HgCl$_2$.4U.2H$_2$O (**1**) and Ni(NO$_3$).2U.2H$_2$O (**2**), were prepared by mixing equal aqueous solutions of HgCl$_2$ (2.72 g, 0.01 mole) and Ni(NO$_3$)$_2$.6H$_2$O (2.90 g, 0.01 mole) in 25 mL distilled water with a 50 mL volume of urea solution (6.0 g, 0.1 mole) in methanol. The mixtures were stirred for about 12 hours under refluxed system at room temperature *ca*. 25 °C. The amount of the precipitate so formed was increased with increasing with the time. The precipitated complex so formed was filtered off, dried under *vacuo* over anhydrous calcium chloride.

Synthesis of urea complexes at 60 °C temperature

The urea complexes, HgCl$_2$.2U.2H$_2$O (**3**) and Ni(NO$_3$).2U. H$_2$O (**4**), were prepared by a method similar to that of described for the preparation of urea complexes at room temperature. A 25 mL volume of urea solution (6.0g, 0.1 mole) in methanol was mixed with an equal volume of HgCl$_2$ (2.72 g, 0.01 mole) and Ni(NO$_3$)$_2$.6H$_2$O (2.90 g, 0.01 mole) and with 25 mL of distilled water. The mixtures were stirred for about 2 hours and then heated to 60 °C for 6 hours on a water bath under refluxed system. The precipitated products were filtered, dried at 60 °C in an oven for 3 hours.

Measurements

The elemental analysis of carbon and hydrogen contents were performed by the microanalysis unit at Cairo University, Egypt, using a Perkin Elmer CHN 2400 instrument. The conductivities of freshly prepared 1.0×10^{-2} g/5 cm^3 dimethylformamide (DMF) solutions were measured for the soluble urea complexes using Jenway 4010 conductivity meter. Magnetic measurements were performed on the Magnetic Susceptibility Balance, Sherwood Scientific, and Cambridge Science Park-Cambridge-England. The infrared spectra with KBr discs were recorded on a Bruker FT-IR Spectrophotometer (4000–400 cm^{-1}). The thermal studies TG/DTG–50H were carried out on a Shimadzu thermogravimetric analyzer under static air till 800 °C. Scanning electron microscopy (SEM) images and Energy Dispersive X-ray Detection (EDX) were taken in Joel JSM-6390 equipment, with an accelerating voltage of 20 KV. The X-ray diffraction patterns for the urea complexes were recorded on X 'Pert PRO PANanalytical X-ray powder diffraction, target copper with secondary monochromate.

Antibacterial and antifungal activities

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method.[38] Briefly, 100 µl of the best bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately108 cells/mL for bacteria and 105 cells/mL for fungi.[39] 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method.[40,41]

Of the many media so available, National Committee for Clinical Laboratory Standards (NCCLS) recommends Mueller-Hinton agar due to main reasons that: it results in good batch-to-batch reproducibility. Disc diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the NCCLS[42] for evaluating the susceptibility of filamentous fungi to antifungal agents.

Disc diffusion method for yeast developed standard method (M44-P) by the NCCLS.[43] Plates inoculated with filamentous fungi as *Aspergillus Flavus* at 25 °C for 48hours; Gram (+) bacteria as *Staphylococcus Aureus, Bacillus subtilis*; Gram (-) bacteria as *Escherichia Coli, Pseudomonas aeruginosa* they were incubated at 35-37 °C for 24-48 hours and yeast as *Candida Albicans* incubated at 30 °C for 24-48 hours and, then the diameters of the inhabitation zones were measured in millimetres.[38] Standard discs of Tetracycline (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter disc impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control.

The agar so used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhabitation have been determined for susceptible values. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10 µl of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. The area of no growth around the disc is known as a "Zone of inhibition" or "Clear zone". For the disc diffusion, the zone diameters were measured with slipping calipers of the National for Clinical Laboratory Standards.[400]

Agar-based methods such as Etest disk diffusion can be good alternatives because they are simpler and faster than broth the methods.[44,45]
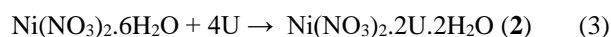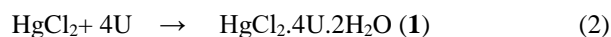
Table 1: Physical characterization, micro-analytical and molar conductance data of urea complexes

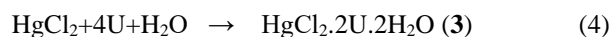| Empirical formula (MW.)	Color	Molar ratio	Elemental analysis (%) Found (Calcd.)		Λm ($\Omega^{-1}cm^2mol^{-1}$)
			C	H	
$HgCl_2.4U.2H_2O$ (**1**) 547.5 g/mol	White	1:4	08.89 (08.78)	03.22 (03.91)	13
$Ni(NO_3)_2.2U.2H_2O$ (**2**) 338.85 g/mol	Light green	1:4	07.16 (07.08)	04.50 (03.54)	215
$HgCl_2.2U.2H_2O$ (**3**) 427.5 g/mol	White	1:4	05.68 (05.61)	02.80 (02.80)	-
$Ni(NO_3)_2.2U.H_2O$ (**4**) 320.85 g/mol	Light green	1:4	07.39 (07.48)	04.30 (03.11)	-

Results and Discussion

The color, physical characteristics, micro-analytical data, molar conductance measurements of Hg(II) and Ni(II) urea complexes are given in Table 1. The elemental analysis data of some prepared complexes revealed 1:4 molar ratio (M:U) (where M= Hg(II), Ni(II) and U= urea) are in a good agreement with the general formulas $HgCl_2.4U.2H_2O$ (**1**), $Ni(NO_3)_2. 2U.2H_2O$ (**2**), $HgCl_2.2U.2H_2O$ (**3**) and $Ni(NO_3)_2. 2U. H_2O$ (**4**). It is important to mention that complexes **1**, and **2** were prepared at room temperature but complexes **3** and 4 were resulted from the chelation of urea with Hg(II) and Ni(II) ions at 60 °C with molar ratio of urea:metal=4:1. The reactions can be represented by the stoichiometric equations:

Reactions at 25 °C:

$$HgCl_2 + 4U \rightarrow HgCl_2.4U.2H_2O \text{ (1)} \qquad (2)$$

$$Ni(NO_3)_2.6H_2O + 4U \rightarrow Ni(NO_3)_2.2U.2H_2O \text{ (2)} \qquad (3)$$

Reactions at 60 °C

$$HgCl_2 + 4U + H_2O \rightarrow HgCl_2.2U.2H_2O \text{ (3)} \qquad (4)$$

$$Ni(NO_3)_2.6H_2O + 4U \rightarrow Ni(NO_3)_2.2U.H_2O \text{ (4)} \qquad (5)$$

The complexes are air-stable, hygroscopic, with low melting points, soluble in H_2O and dimethylformamide. The conductivities of 10^{-2} g/5 ml solutions of the prepared complexes in DMF (Table 1) indicate that the complexes have an electrolytic nature.

Conductivity measurements

The conductivity values for the urea complexes in DMF solvent (10^{-2} g/5 ml solution) are 13 and 215 Ω^{-1} cm^2 mol^{-1} for complexes (**1**) and (**2**) respectively suggesting them to be electrolytes (Table 1). Conductivity measurements have frequently been used in structures of metal complexes (mode of coordination) within the limits of their solubility. They provide a method of testing the degree of ionization of the complexes, the molar ions that a complex liberates in solution (in case of presence anions outside the coordination sphere), the higher will be

its molar conductivity and vice versa. It is clear from the conductivity data that the complex (**1**) seems to be more electrolyte in nature than complex (**2**).

Also the molar conductance values indicate that the anions present outside the coordination sphere. This result was confirmed from the elemental analysis data where Cl^- or NO_3^- ions are precipitated or colored by adding of $AgNO_3$ or $FeSO_4$ solutions, respectively, this experimental test is a good matched with CHN data. Both these complexes have electrolytic properties. This fact elucidated that the Cl^- or NO_3^- are present. These results establish the stoichiometry of these complexes, which are in agreement with the general formulas were suggested.

Magnetic measurements

Magnetic measurements were carried out on a Sherwood Scientific magnetic balance according to the Gauy method. The calculations were evaluated by applying the following equations:

$$\chi_g = \frac{cl(R - R_O)}{10^9 M} \qquad (6)$$

$$\chi_m = \chi_g MWt \qquad (7)$$

$$\mu_{eff} = 2.828\sqrt{\chi_m T} \qquad (8)$$

where

χ is mass susceptibility per gm sample,

c is the calibration constant of the instrument and equal to 0.0816

R is the balance reading for the sample and tube

R_o is the balance reading for the empty tube

M is the weight of the sample in gm

T is the absolute temperature

The magnetic moments of $Ni(NO_3)_2.2U.2H_2O$ (**2**) and $Ni(NO_3)_2.2U.H_2O$ (**4**) complexes at T= 300 K and were calculated. The observed values of the effective magnetic moments μ_{eff} measured were found to be 3.73 B. M. and 2.96 BM. for complexes (**2**) and (**4**), respectively, this is convenient with experimental values of 3.32 B.M. obtained [46] for octahedral Ni(II) complexes with sp^3d^2 hybridization for both Ni(II)/U complexes.

Infrared spectra

The infrared spectra of the Hg(II) and Ni(II) urea complexes at room temperature and at 60 °C are shown in Fig. 1. The band locations were measured for the mentioned urea complexes, together with the proposed assignments for the most characteristic vibrations are presented in Tables 2 and 3. In order to facilitate the spectroscopic analysis and to put our hand on the proper structure of the prepared complexes, the spectra of the urea complexes were accurately compared with those of the urea and similar complexes in literature. The discussion of the spectra will be addressed on the basis of the most characteristic vibrations.

Table 2: Characteristic infrared frequencies (cm^{-1}) and tentative assignments of urea (**U**), [Pt(urea)$_2$Cl$_2$] (**A**), [Cr(urea)$_6$]Cl$_3$ (**B**), HgCl$_2$.4U.2H$_2$O (**1**) and Ni(NO$_3$)$_2$.2U.2H$_2$O (**2**) complexes

Urea	A	B	1	2	Assignments[(b)]
3450	3390	3440	3462	3460	ν(OH); H$_2$O
	3290	3330	3357	3333	ν_{as} (NH$_2$)
3350	3130	3190	-	3361	ν_s(NH$_2$)
	3030				
			1613	1628	δ(H$_2$O)
1683	1725	1505	1667	1660	δ(C=O)
1471	1395	1505	1447	1462	ν(C–N)

The assignments of full vibrational analysis of crystalline urea have been published.[34] Tables 2 and 3 give diagnostic infrared peaks of the free urea ligand, published work Hg(II) and Ni(II) complexes. Assignments have been given in comparison with the data obtained for the free urea, that is, uncoordinated, U and its [Pt(urea)$_2$Cl$_2$] and [Cr(urea)$_6$]Cl$_3$ complexes.[34] The effect of the coordination on the spectra of the complexes of urea with [Pt(urea)$_2$Cl$_2$] and [Cr(urea)$_6$]Cl$_3$ complexes in which the coordination occurs through nitrogen and oxygen atoms, respectively.[34] The mode of coordination of urea with metal ions seems to be dependent upon the type and nature of metal. Pt(II) ions in [Pt(urea)$_2$Cl$_2$] coordinate to the nitrogen, whereas Fe(III), Zn(II), and Cu(II) coordinate to the oxygen of urea.[34]

Table 3: Characteristic infrared frequencies (cm^{-1}) and tentative assignments of urea (**U**), [Pt(urea)$_2$Cl$_2$] (**A**), [Cr(urea)$_6$]Cl$_3$ (**B**), HgCl$_2$.2U.2H$_2$O (**3**) and Ni(NO$_3$)$_2$.2U.H$_2$O (**4**) complexes

Urea	A	B	3	4	Assignments[(b)]
3450	3390	3440	3462	3454	ν(OH); H$_2$O
	3290	3330	3358	3347	ν_{as} (NH$_2$)
3350	3130	3190	2361	3233	ν_s(NH$_2$)
	3030				
			1617		δ(H$_2$O)
1683	1725	1505	1668	1655	δ(C=O)
1471	1395	1505	1448	1473	ν(C–N)

For all the prepared complexes, the coordination mode take place *via* oxygen of amide group, the positively charged metal ion stabilizes the negative charge on the oxygen atom; the NCO group now occurs in its polar resonance form and the double bond character of the CN bond increases or still not affected, while the double bond character of the CO bond decreases, resulting in an
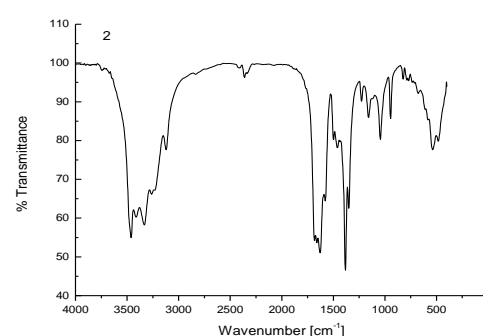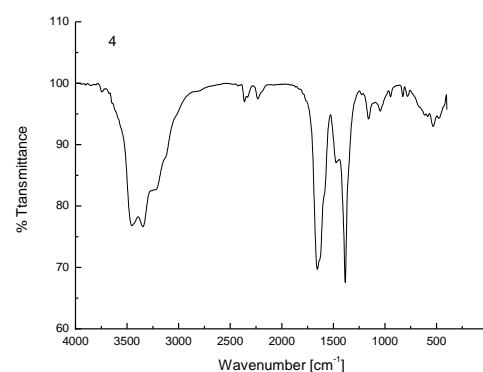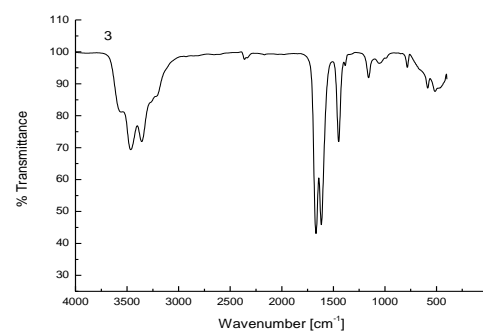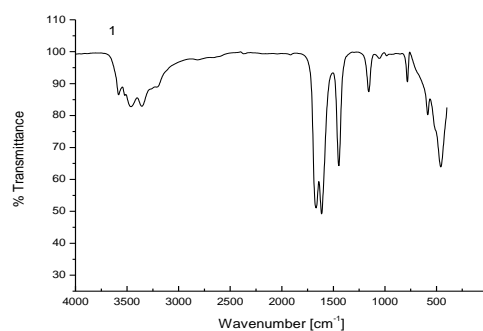

Figure 1a: Infrared spectra of urea complexes HgCl$_2$.4U.2H$_2$O(**1**), Ni(NO$_3$)$_2$.2U.2H$_2$O(**2**), HgCl$_2$.2U.2H$_2$O(**3**) and Ni(NO$_3$)$_2$.2U.H$_2$O(**4**).

increase of the CN stretching frequency with a simultaneous decrease in the CO stretching frequency.[47,48] The IR-active ν(M–O) vibration of all prepared urea complexes is observed at the range (637- 479) cm^{-1}.[47,48] The band related to the stretching vibration ν(O-H) of non-coordinated H$_2$O is observed as expected in the range of (3454-3460) cm^{-1}, while the corresponding bending motion of the non-coordinated water, δ(H$_2$O), is observed in the range of (1613-1628) cm^{-1}.

Eur. Chem. Bull. **2012**, *1(6)*, 188-195

191

Table 4: The maximum temperature, $T_{max}/^{o}C$, and weight loss values of the decomposition stages for the $HgCl_2.4U.2H_2O$(**1**), $Ni(NO_3)_2.2U.2H_2O$(**2**), $HgCl_2.2U.2H_2O$ (**3**) and $Ni(NO_3)_2.2U.H_2O$ (**4**) complexes.

Complexes	Decomposition	$T_{max}/^{o}C$	Lost	% Weight loss	
			Species	Found	Calc.
	Second step	537	$Hg + Cl_2$	51.190	49.605
2	First step	204	$2H_2O$	10.905	10.624
	Second step	332	$NH_3+Urea+N_2O_6$	59.134	41.656
	Third step	501	$NHCO$	11.866	12.699
	Residue		Ni	18.095	17.320
3	First step	248	$2Urea + 2H_2O + Cl_2$	54.917	53.099
	Second step	537	Hg	43.750	46.922
4	First step	108	H_2O	7.995	5.610
	Second step	253	$2\ Urea+2NO_2+\frac{1}{2}O_2$	72.072	71.061
	Third step	440	$0.5O_2$	3.276	4.986
	Residue		Ni	16.657	18.292

In both the nickel complexes, the characteristic stretching vibrations of the nitrato group, NO_3^-, is observed at around (1385 and 1156 cm^{-1} attributed to $v_{as}(NO_3)$ and $v_S(NO_3)$, respectively.[49] The stretching motion of ($v(N=O)$) is observed at 1477 cm^{-1} as a strong band, while the bending motion of the type $\delta(NO_3)$ are well resolved and observed at around 784 cm^{-1} as a medium band.

Thermal analysis

Urea complexes; $HgCl_2.4U.2H_2O$ (**1**), $Ni(NO_3)_2.2U.2H_2O$(**2**), $HgCl_2.2U.2H_2O$ (**3**) and $Ni(NO_3)_2.2U. H_2O$ (**4**) were studied by thermogravimetric analysis from ambient temperature to 800 ^{o}C in oxygen atmosphere. The TG curves and decomposition stages obtained for these complexes with a temperature rate of 30 $^{o}C/min$ are given in the Supplementary material.

Thermal analysis of $HgCl_2.4U.2H_2O$ (**1**) complex (Supplementary material) shows that the mercury(II) complex prepared at room temperature is thermally stable up to 140°C. Its thermal decomposition occurs in two stages of maximum temperatures $DTG_{max}=273$, and 537 ^{o}C, respectively. From the TG-DTG curves (Supplementary material) it is clear that the first decomposition stage corresponds to the loss of 2 mol of water and 4 mol of urea. The continued loss decomposition in the second step corresponds release of Cl_2 and sublimation of mercury or $HgCl_2$ in itself. The probable thermal decomposition of urea released from the complex may be represented as:
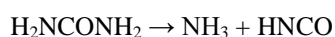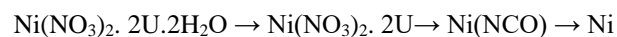
$$H_2NCONH_2 \rightarrow NH_3 + HNCO$$

The mechanism of the thermal decomposition of the complex is proposed as:

$$HgCl_2.4U.2H_2O \rightarrow HgCl_2 \rightarrow Cl_2 + sublimated\ Hg$$
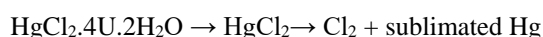
The TG curve of $Ni(NO_3)_2.2U.2H_2O$ (**2**) complex can be shown in the Supplementary material. Three stages of the dissociation of the complex are indicated in TG and

DTG curve. The first decomposition starts at 178 ^{o}C and ends at 261 ^{o}C, with an experimental mass loss of 10.905% against a theoretical loss of 10.624%, this corresponds to the release of 2 molecules of water. The second stage takes place between 261 and 405 ^{o}C, with an experimental mass loss of 59.134 % against a theoretical loss of 59.138 %, which corresponds to the release of 1 mol of ammonia, 1mol of urea and N_2O_4 . The last third stage is from 405 to 561 ^{o}C with maximum peak at 501 ^{o}C, with an experimental mass loss of 11.866 % against a theoretical mass loss of 12.699 %, corresponds to the dissociation of coordinated $NHCO$ leaving Ni as a residue. The mechanism of the thermal decomposition of the complex is proposed as:

$$Ni(NO_3)_2. 2U.2H_2O \rightarrow Ni(NO_3)_2. 2U \rightarrow Ni(NCO) \rightarrow Ni$$

The TG curve of $HgCl_2.2U.2H_2O$ (**3**) complex can be seen in the Supplementary material. The TG-DTG curve indicates that the dissociation of complex is in two stages. The first transition takes place between 137 and 287 ^{o}C, with $DTG_{max}= 248$ ^{o}C, corresponds to the release of 2 mol of urea, 2 mol of H_2O and Cl_2. The second transformation is from 287 to 453 ^{o}C, is due to the sublimation of mercury. The mechanism of the thermal decomposition of the complex is proposed as:

$$HgCl_2.2U.2H_2O \rightarrow Hg \rightarrow sublimated\ Hg$$
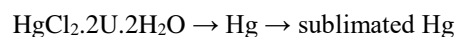
The TG-DTG curve of $Ni(NO_3)_2. 2U.H_2O$ (**4**) complex is also shown in the Supplementary material. Three transition stages of are observed in the TG-DTG curve. The first transition stage is from 46 ^{o}C to 205 ^{o}C with $DTG_{max}= 108$ ^{o}C, with an experimental mass loss of 7.996% against a theoretical loss of 5.610%, corresponding to the release of 1 mol of water . The second transition stage starts at 205 ^{o}C and ends at 329 ^{o}C, with DTG_{max} at 253 ^{o}C with an experimental mass loss of 72.072% against a theoretical mass loss of 71.061%, due to the release of 2 mol of urea, $2NO_2$ and 0.5 O_2. The last and final transition stage which takes place between 358 ^{o}C and 509 ^{o}C is due to the loss of $0.5O_2$ leaving Ni as a residue, the experimental and theoretical mass losses of this stage were 3.276% and 4.986% respectively. The

sequential thermal dissociation process of the complex can be is shown as follows:

$$Ni(NO_3)_2 \cdot 2U \cdot H_2O \rightarrow Ni(NO_3)_2 \cdot 2U \rightarrow NiO \rightarrow Ni$$
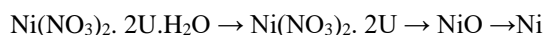
To clarify the final decomposition products of urea complexes, the solid residues after thermal degradation under oxygen atmosphere were collected at 800 ºC and were analyzed by infrared spectroscopy, X-ray diffractometry, EDX and SEM.

X- ray powder diffraction studies

The x-ray powder diffraction patterns for the Hg(II) and Ni(II) urea at different temperatures with formulas; $HgCl_2 \cdot 4U \cdot 2H_2O$(**1**), $Ni(NO_3)_2 \cdot 2U \cdot 2H_2O$ (**2**), $HgCl_2 \cdot 2U \cdot 2H_2O$ (**3**) and $Ni(NO_3)_2 \cdot 2U \cdot H_2O$ (**4**) are depicted in Fig. 3. Inspecting these patterns, we notice that all the systems are well crystalline in nature. The crystal size of these complexes could be estimated from XRD patterns by applying FWHM of the characteristic peaks using Deby-Scherrer equation 1[50]. Where D is the particle size of the crystal gain, K is a constant (0.94 for Cu grid), λ is the x-ray wavelength (1.5406 Å), θ is the Bragg diffraction angle and β is the integral peak width. The particle size was estimated according to the highest value of intensity compared with the other peaks. These data gave an impression that the particle size located within nano scale range.

$$D = K \frac{\lambda}{\beta \cos \theta} \qquad (1)$$

SEM and EDX studies

Scanning electron microscopy is a simplest tool used to give an impression about the microscopic aspects of the physical behavior of urea as a chelating agent (Fig. 3). Although this tool is not a qualified method to confirm complex formation but it can be a reference to the presence of a single component in the synthetic complexes.

The pictures of the Hg(II) and Ni(II) complexes show a small particle size with a nano feature products. The chemical analysis results by EDX for the formed complexes show a homogenous distribution between metal ions and chelating agent. SEM examinations were checked the morphology of the surfaces of these complexes that show small particles which have a tendency to agglomerates formation with different shapes comparison with the start materials. The peaks of EDX profile of these complexes (Fig.5) refer to all elements which constitute the molecules of urea complexes (**1-4**) that clearly identified confirming the proposed structures.
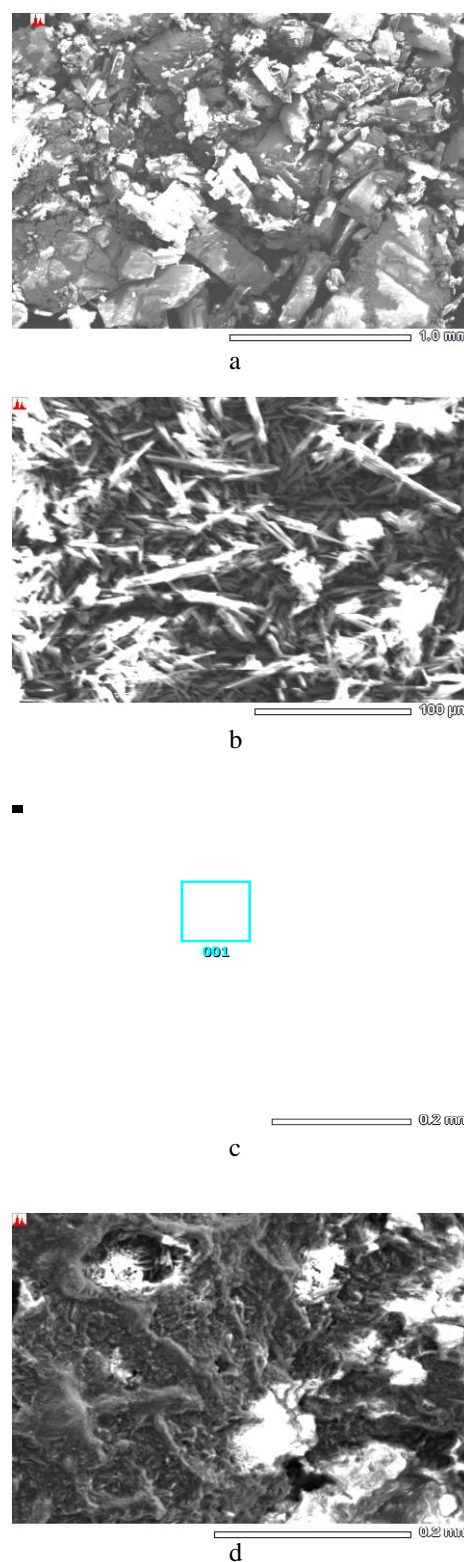

Figure **4**: SEM image of: $HgCl_2 \cdot 4U \cdot 2H_2O$(**1**), $Ni(NO_3)_2 \cdot 2U \cdot 2H_2O$ (**2**), $HgCl_2 \cdot 2U \cdot 2H_2O$ (**3**) and $Ni(NO_3)_2 \cdot 2U \cdot H_2O$ (**4**). complexes

Table 5: Inhibition zone diameter (mm) of the target compounds against tested microorganisms for HgCl₂.4U.2H₂O (**1**) and Ni(NO₃)₂.2U.2H₂O (**2**) complexes

Sample		Inhibition zone diameter (mm / mg sample)					
		Bacillus subtilis (G⁺)	*Escherichia coli* (G⁻)	*Pseudomonas aeruginosa* (G⁻)	*Staphylococcus aureus* (G⁺)	*Aspergillus flavus* (Fungus)	*Candida albicans* (Fungus)
Control: DMSO		0.0	0.0	0.0	0.0	0.0	0.0
Standard	Antibacterial agent (Tetracycline)	36	31	35	30	--	--
	Antifungal gent Amphotericin B	--	--	--	--	18	19
1		41	36	40	37	42	39
2		13	11	14	13	10	22

G: Gram reaction.   Solvent: DMSO.

3-8- Biological evaluation

Biological evaluations were checked in terms of antimicrobial activities of target compounds against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two strains of fungi (*Aspergillus flavus* and *Candida albicans*). Result from the agar disc diffusion tests for antimicrobial activities of target compounds are presented in Table 5, and illustrated in Fig. 4.
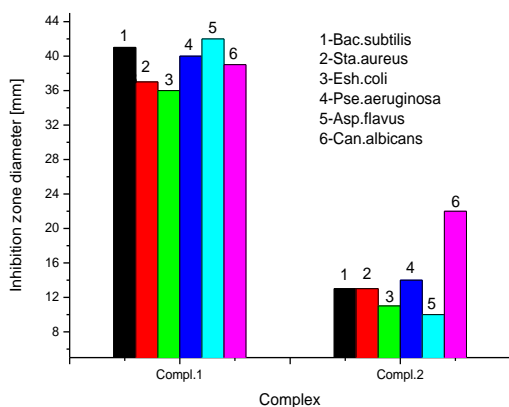
Figure 4 : Biological evaluation of HgCl₂.4U.2H₂O (**1**) and Ni(NO₃)₂.2U.2H₂O(**2**) complexes

The diameters of zone of inhibition (in mm) of the standard drug tetracycline against gram positive bacteria *B. subtilis* and *S. aureus* and gram negative bacteria *E. coli* and *P. aeruginosa* were found to be 36, 30, 31 and 35 mm, respectively, while the standard drug amphotericin B against *Aspergillus flavus* and *Candida albicans* gave 18 and 19, respectively. Under identical conditions, Table 5 shows that, complex **1** has (41, 36, 40, 37, 42 and 39 mm) while complex **2** has (13, 11, 14, 13, 10 and 22 mm)

respectively, for *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus* and *Candida albicans*. Both the complexes were found to be an efficient microbial agents.

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