Investigation on the structural and optical properties of $Zn_{1-x}Cu_xO$ (x=0, 0.05, 0.1) sintered films for optoelectronic device applications

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Keywords: Thick films; Screen printing; XRD; SEM; Raman analysis.

The pure and copper doped zinc oxide $Zn_{1-x}Cu_xO$ (x=0,0.05,0.1) thick films were deposited on glass substrates by screen printing method from their nano powders, followed by sintering at 500 ^{0}C to obtain desired stoichiometry and better adherence of films. The structural and optical properties of the samples were studied by X-ray diffraction (XRD), scanning electron microscopy (SEM) with UV–visible spectroscopy and Raman spectroscopy. XRD patterns confirmed hexagonal wurtzite structure with minor detection of Cu and SEM micrographs revealed granular grains and porosity in films. The optical properties and the energy band gap of pure and Cu^{2+}ions doped ZnO films were studied by UV–visible absorbance spectroscopy. As the doping concentration is increased, both the absorption edge and the reflectance edge is found to shift towards higher wavelengths (red shift) and the direct band gap decreased from 3.4 to 3.3 eV. The incorporation of copper in ZnO lattice is confirmed by Raman spectrum. The E_2 (high) phonon and multiphoton modes are observed at 441 and 1132 cm^{-1} respectively in Raman spectra.

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Introduction

Semiconductor ZnO has attracted extensive experimental and theoretical research efforts for its versatile applications and peculiar properties in transparent electronics, chemical sensors, ultraviolet (UV) light emitters, piezoelectric devices and spintronics since past two decades. Zinc oxide (ZnO) is one of the extensively used material in the field of optoelectronics device applications due to its wide direct band gap of 3.37 eV and a large exciton binding energy of 60 meV as compared to other semiconducting materials, viz., ZnSe (22 meV) and GaN (25 meV). ZnO possess high electrical conductivity, non-toxic nature, high mechanical stability, low cost and higher optical gain (320 cm^{-1}) at ambient temperature which may be controlled by selecting either the non-stoichiometry and by adding suitable dopants.

Doping by other elements (e.g., Al, Ni, Mn, Pd, Cu, Fe, Cd, Ag, Au, etc.) is an adept way to coordinate the band gap of ZnO. It is known that group Ib metals (Cu, Ag, Au) are fast diffusers in compound semiconductors.[1,2] Among these, Cu is the best choice because the size mismatch between Cu and Zn is the smallest which leads to the lowest formation energy. In addition, electrons can be easily injected from Cu layer to ZnO since there is no barrier to the flow of electrons between Cu and ZnO.[3] The diffusion of Cu into ZnO can cause the formation of various centers (Cu_{Zn}, Cu_i). It is possible that Cu atoms can replace either substitutional or interstitial Zn atoms in the ZnO lattice creating structural deformations.[4,5] Cu significantly affects the electrical, chemical, structural and optical properties of ZnO, and the study of the electronic state of Cu in ZnO has been the subject of interest for a long time.[6-9]

Various techniques for the preparation of ZnO films have been used such as electrochemical deposition,[10] cathodic electrodeposition,[11] radio-frequency sputtering method,[12] pulsed laser deposition,[13,14] RF magnetron sputtering,[15] metal organic chemical vapor deposition method,[16] atomic layer deposition,[17] ultrasonic spray pyrolysis,[18] filtered vacuum(cathodic) arc deposition,[19] screen printing,[20] etc. In this work, we have employed fast emerging screen printing technique to deposit ZnCuO thick films. Screen printing technique has been used as a multifaceted method for the fabrication of semiconductor layers in photovoltaic devices, especially II–IV compound semiconductors.[21,22] Compared to the other expensive methods, screen printing is very easier, quicker, eco-friendly and provides a reliable method for film preparation on large area substrates with maximum utilization.

Present work deals with the optimization of synthesis process and parameters for Cu^{2+} ions doped ZnO thick films derived by screen printing technique and characterizing the variation in their structural and optical properties with increase in copper ions concentration in ZnO. The present work aims to deposit ZnCuO thick films by screen printing technique and investigate their structural and optical properties to use them in optoelectronic device applications.

Experimental setup and measurements

Thick film paste was prepared by mixing thoroughly of pure ZnO and CuO (99.999 %) powders with anhydrous $ZnCl_2$ adhesive agent, and ground in a mortar with ethylene

glycol as a binder. The prepared paste was screen-printed on pre-cleaned glass substrates. The pre-cleaning process involves the washing of glass plates with acetone and deionized water, followed by drying at 60 ^{0}C for10 min. The screen-printed films were dried at 110 ^{0}C for2 hours for partial reduction of solvent and inducing porosity in the film[23]. The films were further annealed in a muffle furnace in an open atmosphere at 550 ^{0}C for10 minutes so as to stabilize the films and burn the organic materials. The schematic of screen printing process used for deposition of the thick film is presented as per[24] and is shown in Figure1.

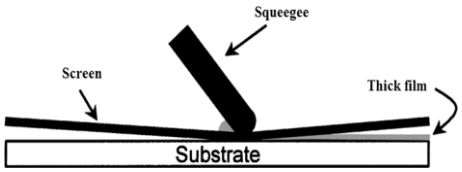


Figure 1. Screen-printed diagram

X-ray diffraction pattern was recorded on advanced Rigaku diffractometer in the 2 h range of 20–70^0 using Cu-$K_{\alpha,}$ X-ray radiation source. The surface morphological information was derived by using scanning electron microscope (SEM, LEO-440, UK). Taylor Hobson (Taly step the UK) instrument was used for film thickness measurement that is of the ordered 4µm. The Raman spectrum was recorded using a Horiba Jobin–Yvon laser Spectrometer 6400. The optical absorption spectrum was recorded by Hitachi Spectrometer-3900 in 200–1000 nm

Results and Discussions

XRD analysis

The typical XRD diffraction peaks of pure ZnO and Cu^{2+} ions doped ZnO ($Zn_{1-x}Cu_xO$) powders with (x = 0, 0.05 and 0.10) are shown in Figure 2.

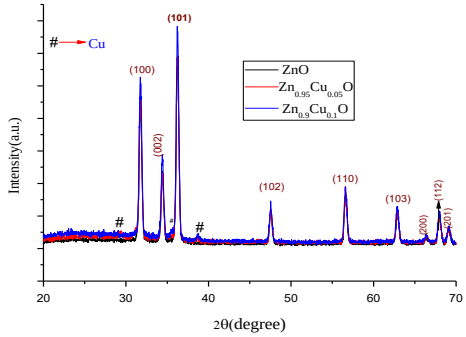


Figure 2. XRD analysis of ZnO, $Zn_{0.95}Cu_{0.05}O$ and $Zn_{0.9}Cu_{0.1}O$

The pronounced diffraction peaks in XRD pattern indicates the polycrystalline nature with peaks corresponding to (100), (002), (101), (102) and (110) planes as per JCPDS data card No. 65-3411[25]. Besides, minor Cu detection is observed at 28^0, 35^0 and 38^0. The standard diffraction peaks confirm the hexagonal wurtzite structure with space group P63mc of Zn_{1-x} Cu_xO, with preferred orientation along (101) plane in all the samples, which is the most stable phase of ZnO. XRD patterns of Cu^{2+} doped ZnO revealed that the decrease in peaks intensities with the increase in the Cu^{2+}concentration suggests the successful substitution of Cu^{2+} ions at Zn lattice site rather than interstitial one. This is due to the fact that ionic radius of Cu^{2+}(0.73Å) is very close to that of Zn^{2+}(0.74Å), due to which Cu can easily penetrate into ZnO crystal lattice. The particle size has been calculated from Debye–Scherer's formula that varies from 26nm to 29nm, this is to due agglomeration of particles.[26]

SEM analysis

Scanning electron microscopy is a convenient method for studying the microstructure of thick films. Figure 3 shows SEM micrographs of as deposited pure and copper doped ZnO thick films sintered at 500 ^{0}C.

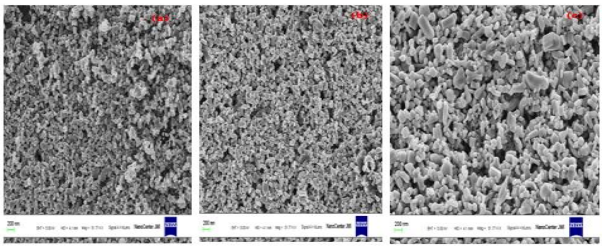


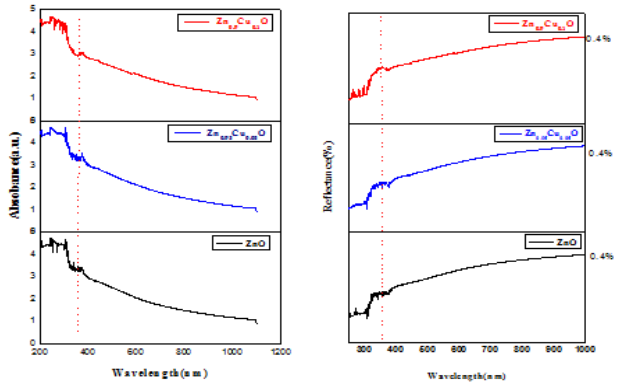
Figure 3. SEM analysis of Cu doped ZnO films; 3(a) pure ZnO. 3(b) $Zn_{0.95}Cu_{0.05}O$. 3(c) $Zn_{0.9}Cu_{0.1}O$

The polycrystalline structure is revealed from the SEM micrographs. The films are porous as evident from the absence of close packed morphology and interconnected grains morphology. The formation of submicrometer crystallites of varying sizes indicates agglomeration in the form of a spindle, cuboidal shaped particles with fused clusters on surface morphology in pure as well as Cu^{2+}ions doped ZnO thick films.

The agglomeration of small crystallites in some regions of the films makes it difficult to find the exact grain size from SEM images. The difference in surface morphology has been observed for Cu:ZnO films as compared to pure ZnO The morphology seems to become a bit rough for Cu:ZnO thick films. It has been observed that increase in copper ion doping concentration in ZnO leads to an increase in the crystallite size which improves with the increase in mobility of atoms at the surface of films. These results also support XRD data. Hence, such types of thick films provide a novel platform for electronic device application.

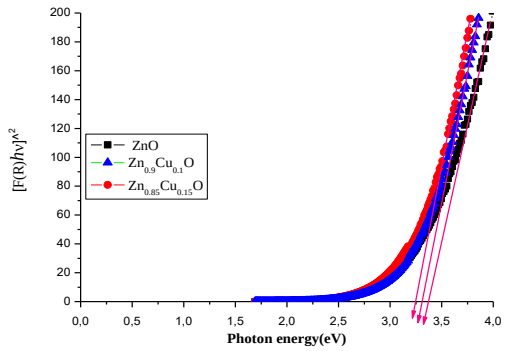
UV-Visible analysis

The optical properties and the energy band gap of pure and Cu^{2+}ions doped ZnO films were studied by UV–visible absorbance spectroscopy in the wavelength range 200-1200 nm and reflectance spectroscopy in the wavelength range 200–1000 nm as shown in Figure4a and Figure4b respectively. As the doping concentration increases, both the absorption edge and the reflectance edge is found to shift towards higher wavelengths (red shift). These peaks arise from the excitation of electron valence band to conduction band and are used to determine the nature and value of optical band gap.



a) b)

Figure 4. Absorption (a) and reflectance (b) spectra of ZnO, $Zn_{0.95}Cu_{0.05}O$ and $Zn_{0.9}Cu_{0.1}O$



c)

Figure 5. Estimation of optical band gap using plot of $[F(R)hv]^2$ versus photon energy of ZnO, $Zn_{0.95}Cu_{0.05}O$ and $Zn_{0.9}Cu_{0.1}O$

The optical band gap of the film is obtained by extrapolation of the linear portion of the graph of the modified Kubelka-Munk function $[F(R)hv]^2$ versus photon energy (hv), given by the following relation.[27]

$$F(R) = \frac{(1-R)^2}{2R} \qquad (3)$$

where R is the magnitude of the reflectance as the function of energy. The direct band gap comes out to be 3.40 to 3.30 eV according to[28] and is shown in Figure 5. This equation is usually applicable for the materials which have high light scattering and absorbing particles in their matrix. Therefore, the reflectance is effective for determining the band gap of the solar cell absorbers.

Raman spectroscopy

Raman spectroscopy technique has been extensively used for characterizing phonon spectra of nanoscale and bulk semiconducting materials. ZnO has wurtzite structure with C_{6v}^4 space group with two molecules per unit cell where all atoms have C_{3v} site symmetry.[29–32] Group theory predicts, $A_1 + 2E_2 + E_1$ is the Raman active phonon modes where A_1 and E_1 are polar phonon modes[31] and E_2 is nonpolar phone mode with two frequencies E_2 (low) is associated with Zn sub-lattice vibrations and E_2 (high) has been related to oxygen bonded vibrations.

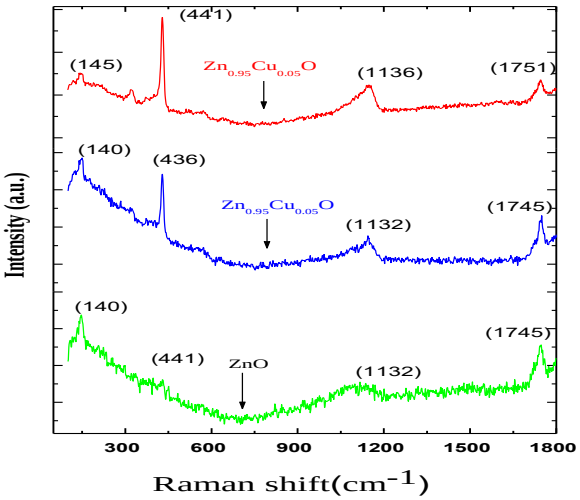


Figure 5. Raman analysis of ZnO, $Zn_{0.95}Cu_{0.05}O$ and $Zn_{0.9}Cu_{0.1}O$

Raman spectra of pure ZnO and copper doped ZnO analogs recorded in 200–1800 cm^{-1} region at ambient temperature are presented in Figure.5 The Raman spectrum shows a strong, sharp peak at 441 cm^{-1}. It is the significant character of the ZnO with hexagonal wurtzite structure. It corresponds to the vibration mode E_{2H} associated with the vibration of oxygen atom known as Raman-active optical phonon mode which is the strongest mode in the system of wurtzite structure.[33] Moreover, the appearance of a sharp peak at 441 cm^{-1} reflects the presence of induced stress in ZnO wurtzite crystal structure.[34]

Figure 5 also exhibits the strong and asymmetric peaks at 1745 [$2A_1$(LO)], and 1132 [$3A_1$(LO)] cm^{-1} phonon modes due to the confinement of optical phonons[35,36] in nano-sized samples.

The weak peak at 140 cm^{-1} has been observed in all the three spectra due to the induced defects. The appearance of higher order and activation of different bands is due to induced electric charge at grain boundaries with the decrease in intensity by increasing copper doping in ZnO lattice.

Conclusion

In conclusion, the pure and copper doped zinc oxide $Zn_{1-x}Cu_xO$ (x=0,0.05,0.1) thick films were synthesized by screen printing technique**.** The incorporation of the Cu^{2+} ion into ZnO lattice rather than the interstitial was observed from XRD analysis. SEM micrograph showed porosity and agglomeration of particles occurred. From UV–visible absorbance spectroscopy it was observed that as the doping concentration was increased, both the absorption edge and the reflectance edge was found to shift towards higher wavelengths (red shift) and the direct band gap decreased from 3.4 to 3.3 eV. Raman analysis confirmed the incorporation of copper in ZnO lattice along with the observation of high and multiphonon modes.

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SYNTHESIS, SPECTROSCOPIC AND ANTIBACTERIAL STUDY OF ZINC, COPPER AND NICKEL COMPLEXES WITH A NEW DERIVATIVE OF L-ASCORBIC ACID

Salah Mohammed Fizea

Keywords: Synthesis, tetradentate, thiocyanate, metal complexes, analysis.

The metal(II) ion complexes of a tetradentate ligand (**L**), namely bis[O,O-2,4-O,O-6,7-(thiocyanatecarboxylic methylidene)]-L-ascorbic acid are synthesized and characterized by ^{1}H,^{13}C-NMR, elemental analysis (CHN), mass spectroscopy, UV-visible and Fourier Transform infrared (FTIR) methods. This ligand is prepared from the reaction of the L-ascorbic acid and two moles of trichloroacetic acid in basic medium (compound I) with two moles of potassium thiocyanate. The reaction of (**L**) in ethanol with $MCl_2.xH_2O$ gave complexes with the general formula $[M(L)Cl_2]$ (where M= Ni(II), Cu(II) and Zn(II), respectively) characterized by FTIR, UV-Visible, molar conductance, atomic absorption, magnetic susceptibility. The analysis of IR, spectral data of all complexes indicated that (**L**) is bonded to metal(II) ions through the two nitrogen atoms of thiocyanate groups and two oxygen atoms of hydroxyl moieties. The synthesized complexes were proposed to be octahedral in geometry. Among these complexes, the Zn(II) complex demonstrated good antibacterial activity.

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Introduction

L-Ascorbic acid (Vitamin C) is an essential nutritious substance for human body participating in many different biological processes. It is found extensively in various vegetables and fruits and is used clinically in the treatment and prevention of scurvy, drug poisoning, liver disease, allergic reaction, and atherosclerosis.[1]

L-Ascorbic acid is an important antioxidant that protects the skin by scavenging and destroying free radicals and reactive oxygen-derived species.[2] L-Ascorbic acid is also used topically because of its ability to reduce wrinkles by promoting collagen synthesis[3] and its skin-depigmenting activity.[4] Because of these favorable effects, L-Ascorbic acid has long been used in pharmaceutical and cosmetic preparations.[5]

Thiocyanate-ion is known to be an important part in the biosynthesis of hypothiocyanite by a lactoperoxidase.[6-8] Thus the complete absence of thiocyanate[9] or reduced thiocyanate,[10] in the human body, (e.g., cystic fibrosis) is damaging to the human host defense system.[11,12]

Thiocyanate-ion shares its negative charge approximately equally between sulfur and nitrogen. As a consequence, thiocyanate can act as a nucleophile at either sulfur or nitrogen it is an ambidentate ligand. [SCN]− can also bridge two (M−SCN−M) or even three metals (>SCN− or −SCN<).

Experimental evidence leads to the general conclusion that class A metals (hard acids) tend to form N-bonded thiocyanate complexes, whereas class B metals (soft acids) tend to form S-bonded thiocyanate complexes.[13]

Experimental

All of the solvents and chemicals were purchased from commercial vendors and were used without purification.

Physical and spectral measurements

The melting points were uncorrected and measured on electrothermal Stuart apparatus, model SMP30. The metal content of the complexes was measured using atomic absorption technique by Perkin-Elmer 5000. Electrical conductivity measurements of the complexes were recorded at 25 °C for 10^{-3} mol L^{-1} solutions in distilled water using Ltd 4071 digital conductivity meter. Magnetic susceptibility values were obtained at room temperature using the Gouy method, Johnson Mattey, model M_5B-MKs, were performed.

FT-IR spectra were recorded in KBr on Shimadzu-spectrophotometer in the range of 4000-400 cm^{-1}. Electronic spectra in distilled water were recorded using a spectrophotometer type Shimadzu in the range of 200-1100 nm with quartz cell of 1 cm path length. Mass spectrum for the ligand L was obtained by Agilent mass spectrometer, Eager 300 for EA1112. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300-MHz spectrometer in DMSO-d_6. Chemical shifts in ppm relative to internal TMS are reported. Elemental microanalyses were carried out by using Euro Vectro-3000A.

Synthesis of ligand (L)

Firstly, compound **I** was prepared according to literature.[14] Then KSCN (0.20 g, 2 mmol) was added to a solution of compound **I** (0.36 g, 1 mmol). The reaction mixture was stirred for three hours at the room temperature. The solid product was filtered off and washed with EtOH, then recrystallized from MeOH:H_2O mixture (3:1). M. p. 198 °C, yield 80 %.

Table 1. Physical properties and analytical data for the synthesized ligand (**L**) and its complexes.

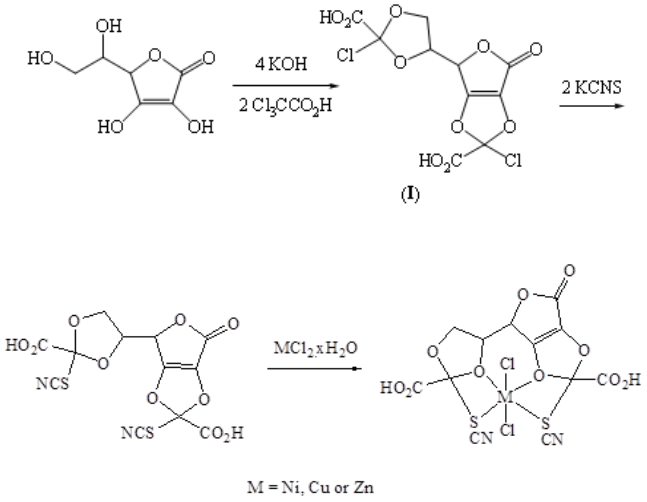
Empirical formula	Color	M.p.° C	Yield, %	Found(Calc.) (%)				
				C	H	N	S	M(II)
Ligand (**L**) $C_{12}H_6N_2O_{10}S_2$	Yellow	198	80	35.57 (35.82)	1.57 (1.50)	6.25 (6.96)	15.34 (15.94)	—
[Cu(**L**)Cl_2]	Green	238	79	26.25 (26.85	1.33 (1.13)	4.98 (5.22)	11.71 (11.95)	11.66 (11.84)
[Ni(**L**)Cl_2]	Olive	251	81	27.77 (27.10)	1.19 (1.14)	5.66 (5.27)	12.35 (12.06)	11.62 (11.03)
[Zn(**L**)Cl_2]	White	248	87	26.17 (26.76)	1.18 (1.12)	5.46 (5.20)	11.78 (11.91)	12.36 (12.14)

Table 2. Magnetic moments and electronic spectral bands (cm^{-1}) of the complexes.

Complex	μ_{eff}, B.M.	Band position, cm^{-1}	Assignments	*B*-complex	β	10Dq (υ_1) theoretical, cm^{-1}
Ligand (**L**)	–	40000	π→π*	–	–	–
L-Ni(II)	3.18	25773 υ_3 14556 υ_2 9174 υ_1	$3A_{2g}\rightarrow3T_{1g}(P)$ $3A_{2g}\rightarrow3T_{1g}(F)$ $3A_{2g}\rightarrow3T_{2g}$	773.38	0.75	13920
L-Cu(II)	2.02	12422	$2Eg\rightarrow2T_{2g}$	–	–	–
L-Zn(II)	Diamagnetic	40983	ILCT	–	–	–

Synthesis of complexes

The ligand (**L**) (0.40 g, 1 mmol) was dissolved in a mixture of 15 mL methanol + 5 ml water. A solution of $NiCl_2.6H_2O$ (475 mg, 2 mmol) in (20 mL) methanol was then gradually added to the solution of (**L**) with stirring to obtain a precipitation accompanied by a visible color changeThe mixture was then refluxed for a further 3 h on a hot plate to ensure the completion of the reaction. The yellow precipitate was then filtered off, washed with methanol. The same procedure was used for the synthesis of the Cu(II) and Zn(II) complexes. The isolated complexes are colored solids, stable in air and insoluble in common organic solvents but completely soluble in water, DMSO and DMF. Some physical properties of synthesized ligand (L) and its complexes are shown in Table 1.



Scheme 1. Preparation of the ligand (L) and its complexes.

RESULTS AND DISCUSSION

Bis[O,O-2,4:O,O-6,7(thiocyanatecarboxylic methylidene)] L-ascorbic acid (**L),** was obtained in a good yield by the reaction of the compound (**I**) with potassium thiocyanate, in the ratio of 1:2. Then the metal complexes were obtained from the reaction of ligand **(L**) with $MCl_2.xH_2O$ in EtOH, having the general formula [M(L)Cl_2] (where M = Ni(II), Cu(II) and Zn(II), respectively; Scheme 1).

The molar conductance of all complexes was obtained to be 16, 21 and 19 Ω^{-1} cm^2 mol^{-1}, respectively. This indicates that all of the three complexes are non-electrolyte.[15] So based on elemental analysis and molar conductance data, all complexes have [M(L)Cl_2] formula in which two chloride ions are placed on coordination sphere.

A single sharp band was observed at 2056 cm^{-1} in IR spectrum of (**L)** that can be assigned to –SCN group, although its position was found to be lower by 25–35 cm^{-1} in its complexes. This behavior suggests that nitrogen atom of the thiocyanate coordinated to the metal(II) ions.

New bands appeared at around 470 and 455 cm^{-1} in all complexes, which were attributed to the ν(M–O) and ν(M–N) stretching vibrations, respectively.[16]The two strong bands at 1722 and 1670 cm^{-1} due to asymmetric and symmetric frequencies of ν(C=O) in (**L**), were observed to shift to 1717–1729 and 1675–1680 cm^{-1} in the spectra of all complexes.[17] Since no considerable change was observed for ν(C=O) of (**L**) and its complexes, it was concluded that the oxygen atoms of the carbonyl in the carboxylic group are not involved in the coordination to the metal ions.

The ^{1}H-NMR spectrum of (**L**) displayed several signals at 4.54 ppm is attributed to CH-6, while CH-5 of lactone ring appears at 6.34 ppm. The proton signal of CH_2-7 appeared at 3.36 ppm, the signal at 8.30 ppm can be assigned to OH of carboxylic acid.

^{13}C-NMR spectrum showed a weak signal at 179 ppm which belongs to the carboxylic acid, while the C=O carbon signal appears at 162 ppm. The two peaks at 129 and 138

ppm are attributed to C-2 and C-4 carbons, respectively. This may be due to the conjugated double bond from C-1 to C-4 causing upfield shift of C-4 carbon signal. The C-N carbon signal of thiocyanate appears at 111 ppm, the signals at 79, 70 and 62ppm assigned to C-5, C-6 and C-7 carbon atoms, respectively.

The mass spectra of the ligand (**L**) exhibit fragmentation patterns as expected. Therefore the results of mass spectroscopy showed good agreement with the molecular weight 402 as expected. The peak for $[M+H]^+$ was observed at *m/z* 403.

The electronic spectra of (**L**) and its complexes show an absorption band in the region 250–260 nm that can be attributed to intra-ligand transfer $\pi\rightarrow\pi^*$ transition.[18] The electronic absorption bands, as well as the magnetic moment values of ligand **L** and its complexes, are summarized in table 2. Spectrum of Ni(II) complex exhibited three bands in the visible region at 25773 cm^{-1} ($^3A_2g\rightarrow{}^3T_1g_{(P)}$) ($\upsilon_3$), 14556 cm^{-1} ($^3A_2g\rightarrow{}^3T_1g_{(F)}$) ($\upsilon_2$) and the last one is at 9174 cm^{-1} can be attributed to $^3A_2g\rightarrow{}^3T_2g$, ($\upsilon_1$). The ratio of υ_2/υ_1, (1.59) was applied on Tanabe-Sugano diagram for d^8 octahedral complexes,[19,20] $B_{\text{-complex}}$ and β, 10*Dq*(υ_1) were calculated theoretically. The spectrum of Cu(II) complex showed a broad band at 12422 cm^{-1} assigned to $^2Eg\rightarrow{}^2T_2g$ transition which refers to Jahn-Teller distortion of octahedral geometry.[21] The Zn-complex is diamagnetic. As it is expected due to the d^{10} electronic configuration of Zn(II) ion, its electronic spectra did not show any d–d transition, only band assigned to charge transfer transition at 40983 cm^{-1}.

Table 3. Antimicrobial activity of ligand **L** and its complexes; diameter of growth of inhibition zone, mm.

Compounds	*S. aureus*	*E. coli*
Ligand (**L**)	0	0
Ni-complex	14	18
Cu-complex	17	21
Zn-complex	20	24

The ligand (**L**) and its complexes have been screened for antibacterial activities. The results of the microbial screening of (**L**) and its complexes are given in table 3. The antibacterial monitoring data showed that ligand (**L**) does not exhibit any activity. While its complexes demonstrated antibacterial activity towards Gram negative bacteria (*E. coli*), and Gram positive bacteria (*S. aureus*). The increased activity of the metal complexes can be described based on chelating theory.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SCHIFF BASES AND THEIR Co(II), Cu(II), Ni(II) CHELATES FROM DERIVATIVE CONTAINING INDOLE MOIETY BEARING-TRIAZOLE RING

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Keywords: Design, synthesis, biological evaluation triazole, indol.

Indole and its various derivatives show diverse biological effects. In the present study, some novel indole derivatives like methyl-2-(1*H*-indole-3-yl)-ethanoate derivatives (**D1-D5**) have been synthesized. Three Schiff bases (**D6-D8**) have been synthesized by the reaction of (**D5**) with substituted benzaldehydes. Co(II), Cu(II) and Ni(II) complexes of the Schiff bases have been prepared. All the compounds have been characterized by elemental analysis, molar conductivity, magnetic moment effect and spectroscopic techniques. The compounds exhibited moderate to significant anti-bacterial and anti-fungal activities against *E. coli*, S. *aurous*, *B. subtitles*, *P. aeruginosin*, *A. niger*, *A. flavours*, R. *stolonifera* and *C. Albicans*
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Introduction

The heterocyclic compounds which have thiazole, indole and tetrazole groups display great scope of biological efficiencies.[1] One of the common but remarkable group of alkaloid compounds is indoles, which appears to have important role in many biochemical reactions. Indole ring constitutes one of the fundamental moiety in the strucutre of the drug such as Indoxole, Tenidap, Etodolac, hormones serotonin, Indomethacin, the psychotropic drug LSD, melatonin, and the antitumour agent vinblastine.[2-4] The indole ring framework is found wide spread in nature. Indole compounds and their derivatives are used in numerous pharmacological activities like antioxidant, antifungal, antipsychotic, anti-inflammatory, cardiovascular activity, antidepressant, antimycobacterial, analgesic, antibacterial activities and anti-HBV. The present research work focuses on the efficient synthesis of novel indole derivatives and their biological evaluation.[5-8]

Schiff bases and their metal complexes have diversified implementations in analytical,[9] biological, pharmacological clinical,[10] and corrosion science areas.[11] In addition they are used in specific chemical reactions as catalysts in hydrolysis,[12] decomposition,[13] oxygenation,[14] electro-reduction,[15] and enzymatic reactions.[16] Metal complexes of ligands with groups like carbonyl or amino have been reported to show higher activity against many diseases[17] as compared to the organic components.[18,19] We report herein the synthesis and biological evaluation of methyl-2-(1*H*-indole-3-yl)-ethanoate derivatives (**D1-D5**), Schiff bases (**D6-D8**) and their Co(II), Cu(II), Ni(II) chelates.

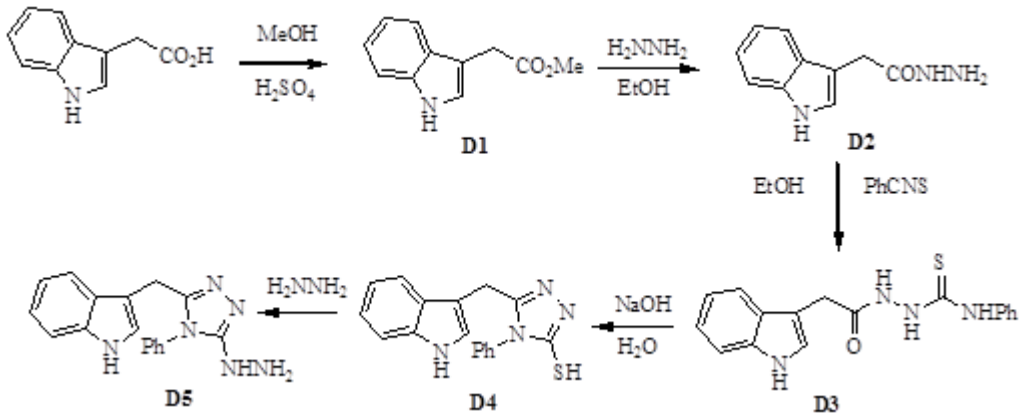
Experimental

Materials

The starting compounds were obtained commercially and used without moreover refining. Indole-3-acetic acid (99 %, Tabtech Chemical), hydrazine hydrate (80 %, Aldrich), diethyl ether (99.5 %, Scharlau), ethanol(99.8 %, Riedel-de Haën), sulfuric acid (98 %, Merck), phenylisothiocyanate (97 %, BDH), sodium hydroxide (97 %, Aldrich), hydrochloric acid (37 %, Riedel-de Haën), 2-hydroxybenzaldehyde (99.8 %, Aldrich), glacial acetic acid (99.8 %, Riedel-de Haën), 2-methoxybenzaldehyde (99.8 %, Aldrich), 2-hydroxy-3-methoxybenzaldehyde (99.5 %, Aldrich), methanol (99.8 %, Riedel-de Haën) cobalt (II) chloride hexahydrate $CoCl_2.6H_2O$ (99 %, Merck), acetone (99.8 %, Riedel-de Haën), nickel(II) chloride hexahydrate (98 %, B.D.H) and copper(II) chloride dihydrate $CuCl_2.2H_2O$ (98%, B.D.H).

Techniques

FTIR spectra were recorded by using potassium bromide discs on a SHIMADZU (IR Affinity-1) FTIR spectroscopy at College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad. ^{1}HNMR spectra were obtained by Ultra Shield 300 MHz, Bruker, at the University of Al-Bayt (in Jordan) while some spectra were recorded on Bruker model, ER-AV-400 MHz, at University of Science and Technology, in Jordan and are reported in ppm (δ). DMSO-d_6 was used as a solvent with TMS as an internal standard. The mass spectra were recorded on Shimadzu model: GCMS QD 1000 EX. Chloride content of the complexes was determined using potentiometric titration method (686-Titro Processor-665 Dosim A-Metrohm/Swiss). UV-Vis spectrophotometer type CECIL, England, was used with quartz cell of 1 cm path length in the range of 200-1000 nm in a 10^{-3} M solution in ethanol at room temperature.



Scheme 1. Synthesis of indol-bearing triazole hydrazines

Magnetic susceptibility measurements were obtained using Bruker BM6 at 298 K. Elemental microanalysis of some compounds performed on a C.H.N analyser Euro vector, model Euro EA 3000A (Italy). The TLC was performed on aluminum plate coated with a layer of silica gel, supplied by Merck. The compounds were detected by iodine vapour.

Preparation of hydrazine compounds

Step 1. A mixture of 1*H*-indole-3-acetic acid (0.123 mol), in absolute methanol (50 mL) and sulfuric acid (1.35 mL) was refluxed for 6 h. After cooling the mixture was washed with sodium bicarbonate solution, then washed with water several times, dried and recrystallized from ethanol to yield methyl- 2-(1*H*-indole-3-yl)-ethanoate (**D1**). Yield 94 %. Anal Calcd. for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40 Found: C, 6; H, 4.94; N, 20.58. IR cm^{-1}, 3408 (NH), 3057 (CH), 2951(CH), 1732 (C=O)

Step 2. A mixture of ester compound (**D1)** (0.003 mol) and 80 % hydrazine hydrate (0.75 mL) in absolute ethanol (1.25 mL) was refluxed for 3 h. The mixture was cooled to room temperature, the solvent was evaporated and the solid formed was recrystallized from diethyl ether to yield 2-(1*H*-indol-3-yl)acetol hydrazide (**D2**). Yield 84 %. m.p = 144-146 ˚C. Anal Calcd. for $C_{10}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.21; Found: 63.48; H, 5.86; N, 22.21. IR (cm^{-1}) 3316, 3278, 3142 (NH, NH_2), 3143, 3057 (CH aromatic), 1681 (C=O amide), 1639 (C=N), 1598, 1550, 1499 (C=C)

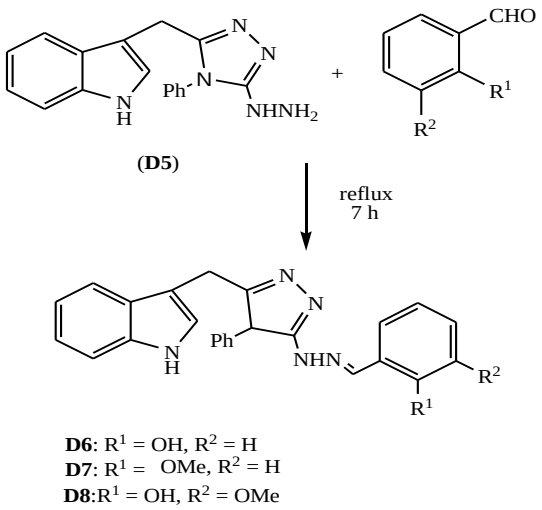
Step 3. A mixture of (**D2**) (0.001 mol) and phenyl isothiocyanate (0.135 g, 0.001 mol) in ethanol (5 mL) was refluxed for 4 h. The reaction mixture was cooled to room temperature and the solid obtained was filtered, dried and recrystallized from methanol to yield 5-((1H-indol-3-yl)methyl)-4-phenyl- 4H-1,2,4-triazole-3-thiol (**D3**). Yield 84 %. m.p = 144-146 ˚C. Anal. Calcd. for $C_{17}H_{16}N_6$: C, 67.09; H, 5.30; N, 27.61; Found: C, 63.48; H, 5.86; N, 27.61. MS *m/z*: 304.14 (100. 0%), 305.15 (18.6 %), 305.14 (2.2 %), 306.15 (1.6 %) IR (cm^{-1}) 3322, 3278, 3142, 1683. (Figure 3). ^{1}H NMR (DMSO-d_6) δ = 13.38 (s, 2H), 8.71 (s, 2H), 8.02 (d, 4H), 7.40 (d, 4H), 7.29 (dd, 4H), 6.90 (t, 2H), 2.35 (s, 6H).

Step 4. A mixture of (**D3**) (0.005mol) and 5 mL of 4% aqueous sodium hydroxide solution was refluxed for 4 h and then treated with 10 % HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethanol to afford 5-[(1*H*-indol-3-yl)methyl)-4-phenyl-1,2,4-triazole-3-thiol (**D4**). Yield 93 %. m.p = 258-260 ˚C. Anal. Calcd. for $C_{17}H_{14}N_4S$: C, 66.64; H, 4.61; N, 18.29; S, 10.47. Found: C, 66.22; H, 4.31; N, 17.81; S, 10.17. MS (*m/z*): 306.0 (100%), 131 (18.5 %), 273, 246 (6.5 %), 229(6.5 %), 206 (6.7 %), 189 (1.3 %), 170(1.8 %), 155 (29.6 %), 130 (44. 7 %), 128 (10.9 %), 103 (11.4 %), 91 (4.8 %) 77 (31.9 %), 65 (7.8 %), 51 (12.5 %). IR (cm^{-1}) 3404-3128 (NH, NH_2), 3047, 3012 (CH aromatic), 2974 (CH aliphatic), 1681 (C=O amide), 1548, 1460 (C=C aromatic), 1276(C=S).

Step-5. To a solution of (**D4**) (0.765 g, 0.0025 mol) in 5 mL of ethanol, 80 % hydrazine hydrate (0.5 mL, 0.01 mol) was added drop wise with stirring and the mixture was refluxed for 2 days, then the excess of solvent was evaporated and the solid recrystallized from chloroform to give the desired white product, of 3-[(5-hydrazinyl-4-phenyl-1, 2, 4-triazol-3-yl)-methyl]- 1*H*-indole (**D5**). The reactions are presented in Scheme 1. Yield 84 %. m.p = 144-146 ˚C, Anal. Calcd. for $C_{17}H_{16}N_6$: C, 67.09; H, 5.30; N, 27.61; Found: C, 66.78; H, 5.11; N, 27.23. MS (*m/z*): 304.14 (100.0 %), 305.15 (18.6 %), 305.14 (2.2 %), 306.15 (1.6 %). IR (cm-1) 3362-3155 (NH, NH2), 3046 (CH aromatic), 2937 (CH aliphatic), 1681(C=O amide), 1515, 1490, 1468 (C=C aromatic) (Figure 5). 1H NMR (DMSO-d6,) □ = 2.50 (s, 6H, DMSO), 3.63 (b, 2H, CH_2), 4.65 (s, 2H, NH2), 6.63-7.47 (m, 13H, CH=), 9.12 (s, 1H, NH), 10.0l (s,1H, NH indole ring)

Synthesis of new Schiff bases (D6-8)

A mixture of primary amino compound (**D5**) (0.025 mol), and a substituted benzaldehyde, 2-hydroxybenzaldehyde, 2-methoxybenzaldehyde or 2-hydroxy-3-methoxy-benzaldehyde (0.025 mol) in absolute ethanol (25 mL) was refluxed for 6 h. The solvent was evaporated under vacuum and the residue was recrystallized from methanol (Scheme 2).



Scheme 2. Synthesis of **D6-D8**.

2-((2-(5-((1*H*-indol-3-yl)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)hydrazono)methyl)-6-methoxyphenol (D6)

Yield 71 %. F. W. 408.46. Anal. Calcd. for $C_{24}H_{20}N_6O$: C, 70.57; H, 4.94; N, 20.58; Found: C, 70.21; H, 4.63; N, 20.10. MS *m/z* 408.17 (100.0 %), 409.17 (28.2 %), 410.18 (3.3 %). IR: 3506 (OH), 3272 (NH), 3031 (CH aromatic), 2972 (CH aliphatic), 1642 (C=N), 152 (C=C aromatic) cm^{-1} (Figure 7). ^1H NMR (DMSO-d_6, 500 MHz) δ = 2.52 (s, 6H, DMSO), 3.35 (s, 3H, OCH_3), 6.93-7.54 (m, 13H, CH=), 8.06 (s, 1H, NH), 10.27 (s, 1H, NH), 10.53 (s, 1H, NH), 11.17 (s, 1H, OH). UV-VIS (DMSO): λ_{max} = 266, 353 nm; ε = 37593, 28328 $M^{-1}cm^{-1}$.

3-((5-(2-(2-Methoxybenzylidene)hydrazinyl)-4-phenyl-4H-1,2,4-triazol-3-yl)methyl)-1*H*-indole (D7)

Yield 71 %. F. W. 422.48. Anal. Calcd. for $C_{25}H_{22}N_6O$: C, 71.07; H, 5.25; N, 19.89; Found: C, 71.05; H, 5.08; N, 19.23; MS *m/z* 422.19 (100.0 %), 423.19 (27.3 %), 424.19 (4.3 %), 423.18 (2.2 %); IR: 3267 (NH), 3042 (CH aromatic), 2968 (CH aliphatic), 1648 (C=N), 1578 (C=C aromatic), 1250 (OCH_3) cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz) δ = 2.50 (s, 6H, DMSO), 3.69 (s, 3H, OCH_3) 3.94 (s, 2H, CH_2), 6.40-7.57 (m, 13H, CH=), 9.32 (s,1H, CH=N), 10.32 (s, 1H, NH), 10.82 (s, 1H, NH). UV-VIS (DMSO): λ_{max} = 247, 382 nm; ε = 40485, 28328 $M^{-1}cm^{-1}$.

2-((2-(5-((1H-indol-3-yl)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)hydrazono)methyl)-6-methoxyphenol (D8)

Yield 71 %. F. W. 438.48 Anal Calcd. for $C_{25}H_{22}N_6O_2$: C, 68.48; H, 5.06; N, 19.17. Found: C, 68.22; H, 4.87; N, 18.84. MS *m/z* 438.18 (100.0 %), 439.18 (29.3 %), 440.19 (3.6 %), 440.18 (1.0 %). IR: 3522 (OH), 3257 (NH), 3082 (CH aromatic), 2973 (CH aliphatic), 1642 (C=N), 1582 (C=C aromatic), 1258 (OCH_3) cm^{-1}. ^1H NMR (DMSO-d_6 500 MHz) δ = 2.50 (s, 6H, DMSO), 3.69 (s, 3H, OCH_3), 3.94 (s, 2H, CH_2), 6.40-7.57 (m, 13H, CH=), 8.91 (s, 1H, CH=N), 9.12 (s,1H,NH), 9.89 (s, 1H, NH), 11.17 (s, 1H, OH). UV-VIS (DMSO): λ_{max} 266, 353 nm; ε = 37593, 28328 M^{-1} cm^{-1}.

Cu, Ni and Co complexes of ligands D6-D8

Ligand (**D6-D8**; 0.02 mmol) and metal salts ($CuCl_2.2H_2O$, $CoCl_2.6H_2O$, and $NiCl_2.6H_2O$; 0.01 mmol) were dissolved in ethanol (15 mL) and the mixture was refluxed for 1.5 h. The mixture was allowed to stand at room temperature for a few days. Then the precipitate formed was filtered and washed with ethanol (2 mL) and diethylether (5 mL) and dried at room temperature.

Co-complex of D6 (S1)

Yield 80 %. F. W. 873.83; Anal Calcd. for $C_{48}H_{38}CoN_{12}O_2$: C, 65.98; H, 4.38; Co, 6.74; N, 19.23. Found: C, 65.65; H, 4.11; Co, 6.53; N, 19.08. IR: 3281(NH), 3068 (CH aromatic), 2982 (CH aliphatic), 1632 (C=N), 1584 (C=C aromatic), 447 (M-O), 559 (M-N) cm^{-1}. UV-VIS (DMSO) λ^{max} = 274, 425, 873, 962 nm; ε = 36496, 23529, 11454, 10395 M^{-1} cm^{-1}.

Ni-complex of D7 (S2)

Yield 77 %. F. W. 873.59. Anal Calcd. for $C_{48}H_{38}N_{12}NiO_2$: C, 65.99; H, 4.38; N, 19.24; Ni, 6.72; . Found: C, 65.43; H, 4.23; Ni, 18.84; Ni, 6.54. IR: 3278 (NH), 3071 (CH aromatic), 2968 (CH aliphatic), 1634 (C=N), 1580 (C=C aromatic), 450 (M-O), 558 (M-N) cm^{-1}. UV-VIS (DMSO) λ^{max} = 279, 364, 682, 981 nm; ε = 35842, 27472, 14662, 10193 $M^{-1}cm^{-1}$.

Cu-complex of D8 (S3)

Yield 64 %. F. W. 878.44. Anal. Calcd. for $C_{48}H_{38}CuN_{12}O_2$: C, 65.63; H, 4.36; Cu, 7.23; N, 19.13; Found: C, 65.23; H, 4.21; Cu, 7.09; N, 18.85. IR: 3283 (NH), 3075 (CH aromatic), 2973 (CH aliphatic), 1630 (C=N), 1576 (C=C aromatic), 441 (M-O), 558 (M-N) cm^{-1}. UV-VIS (DMSO) λ^{max} = 271, 758 nm; ε = 36900, 13192 $M^{-1}cm^{-1}$.

Co-complex of D6 (S4)

Yield 74 %. F. W. 973.81. Anal. Calcd. for $C_{50}H_{43}CoN_{12}Cl_2O_2$: C, 61.67; H, 4.45; Co, 6.05; N, 17.26 Cl, 7.28; Found: C, 6l.20; H, 4.08; Co, 6.12; N, 17.02; Cl, 6.89. C, 65.23; H, 4.21; Cu, 7.09; N, 18.85. IR: 3261 (NH), 3071 (CH aromatic), 2992 (CH aliphatic), 1673 (C=N), 1574 (C=C aromatic), 1239 (OCH_3) cm^{-1}. UV-VIS (DMSO) λ^{max} = 268, 345, 728, 758 nm; ε = 37313, 28985, 14749, 13850 $M^{-1}cm^{-1}$.

Ni-complex of D7 (S5)

Yield 62 %. F. W. 973.57. Anal Calcd. for $C_{50}H_{43}Cl_2N_{12}NiO_2$: C, 61,69; H, 4.45; N, 17.26; Ni, 6.07 ; Cl, 7.26; Found: C, 61.08; H, 3.78; Ni, 16.96; Ni, 5.87; Cl, 6.77. IR: 3268 (NH), 3068 (CH aromatic), 3063 (CH aliphatic), 1634 (C=N), 1571 (C=C aromatic), 1242 (OCH_3), 448 (M-O), 559 (M-N) cm^{-1}. UV-VIS (DMSO) λ^{max} = 262, 328, 682 nm; ε = 38167, 30487, 14662 $M^{-1}cm^{-1}$.

Cu-complex of D8 (S6)

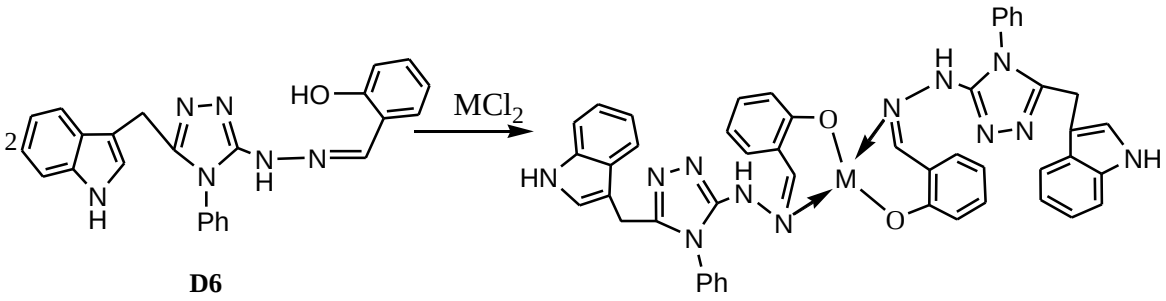
Yield 69 %. F. W. 978.42. Anal Calcd. for $C_{50}H_{43}Cl_2CuN_{12}O_2$: C, 61.38; H, 4.43; Cu, 6.49; N, 1718 Cl, 7.25; Found: C, 65.23; H, 4.21; Cu, 7.09; N, 18.85. IR: 3261 (NH), 3078 (CH aromatic), 2971 (CH aliphatic), 1636 (C=N), 1574 (C=C aromatic), 1240 (OCH_3), 449 (M-O), 560 (M-N) cm^{-1}. UV-VIS (DMSO) λ^{max} = 266, 372, 478 nm; ε = 37593, 26881, 20920 $M^{-1}\,cm^{-1}$.

Co-complex of D6 (S7)

Yield 61 %. F. W. 933.28. Anal Calcd. for $C_{50}H_{42}CoN_{12}O_4$: C, 64.31; H, 4.53; Co, 6.31; N, 18.00; Found: C, 64.11; H, 4.32; Co, 6.15; N, 17.87. IR: 3261 (NH), 3078 (CH aromatic), 2971 (CH aliphatic), 1636 (C=N), 1574 (C=C aromatic), 1240 (OCH_3), 442 (M-O), 551 (M-N) cm^{-1}. UV-VIS (DMSO) λ^{max} = 249, ?, 522, 699, 786 nm; ε = 40160, 28818, 19157, 14947, 12722 $M^{-1}\,cm^{-1}$.

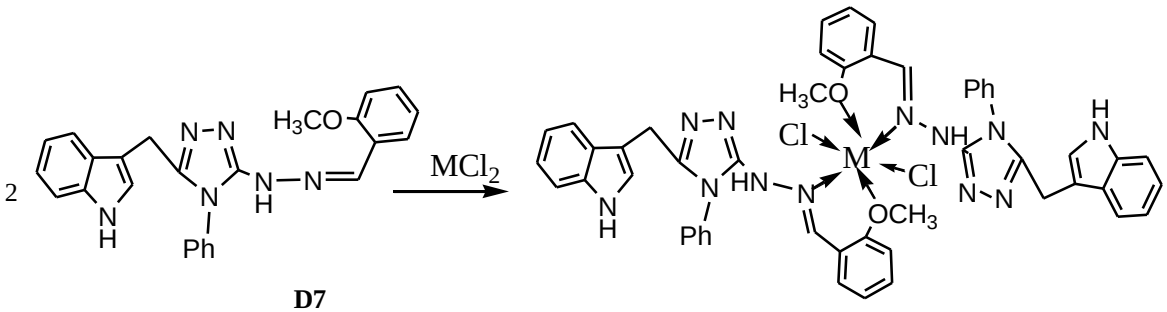
Ni-complex of D7 (S8)

Yield 78 %. F. W. 933.64; Anal Calcd. for $C_{50}H_{42}N_{12}NiO_4$: C, 64.32; H, 4.53; Ni, 6.29; N, 18.00; Found: C, 64.22; H, 4.31; Ni, 6.09; N, 17.82. MS (*m/z*): 438.18 (100.0 %), 439.18 (29.3 %), 440.19 (3.6 %), 440.18 (1.0 %). IR: 3261 (NH), 3078 (CH aromatic), 2971 (CH aliphatic), 1636 (C=N), 1574 (C=C aromatic), 1240 (OCH_3), 458 (M-O), 572 (M-N) cm^{-1}. UV-VIS (DMSO) λ_{max} = 243, 363, 522, 897 nm; ε = 41152, 27548, 19157, 11148 $M^{-1}\,cm^{-1}$



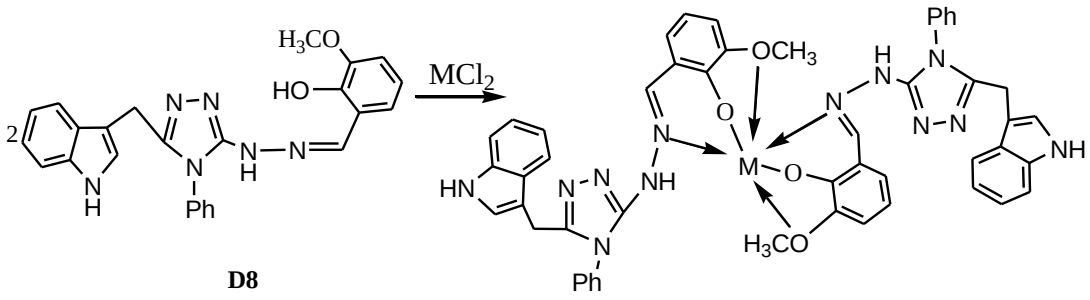
D6

Scheme 3. Formation of metal complexes of **D6**.



D7

Scheme 4. Formation of metal complexes of **D7**.



D8

Scheme 5. Formation of metal complexes of **D8**.

Table 1. Results of antibacterial bioassay (concentration used 100 μg mL^{-1} in DMSO). (a) *E. coli*, (b) *S. aurous* (c) *B. subtilis* (d) *P. aeruginosa*. Antifungal bioassay (concentration used 200 μg mL^{-1}). (a) *A. niger* (b) *A. flavus* (c) *R. stolonifer* and (d) *C. albicans* .10 <: weak; > 10: moderate; > 16: significant.

Compound	Bacteria				Fungus			
	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
D6	8	11	8	9	19	21	23	24
$[Co(D6)_2]$	15	14	16	13	24	26	27	24
$[Ni(D6)_2]$	13	15	15	13	28	23	21	26
$[Cu(D6)_2]$	12	16	14	13	20	23	26	22
D7	5	8	6	12	18	19	17	19
$[Co(D7)_2]$	11	10	7	1l	19	2o	21	20
$[Ni(D7)_2]$	14	12	15	13	26	27	26	27
$[Cu(D7)_2]$	12	14	14	15	19	20	18	20
D8	9	10	12	11	25	25	22	24
$[Co(D8)_2Cl_2]$	13	15	15	13	28	23	21	26
$[Ni(D8)_2Cl_2]$	15	13	1l	16	24	2l	23	27
$[Cu(D8)_2Cl_2]$	17	15	16	14	24	26	29	25

Cu-complex of D8 (S9)

Yield 68 %. F. W. 938.49. Anal Calcd. for $C_{50}H_{42}CuN_{12}O_4$: C, 63.99; H, 4.51; Cu, 6.77; N, 17.91; Found: C, 63.71; H, 3.96; Cu, 6.12; N, 17.65. MS (*m/z*): 438.18 (100. 0%), 439.18 (29.3 %), 440.19 (3.6 %), 440.18 (1.0 %). IR: 3261 (NH), 3078 (CH aromatic), 2971 (CH aliphatic), 1636 (C=N), 1574 (C=C aromatic), 1249 (OCH_3), 461 (M-O), 548 (M-N) cm^{-1}. UV-VIS (DMSO) λ_{max} = 247, 358, 486, 576 nm; ε = 40485, 27932, 19157, 17361 M^{-1} cm^{-1}.

Results and Discussion

3-((5-Hydrazinyl-4-phenyl-4*H*-1,2,4-triazole-3-yl) methyl)-1*H*-indole (**D5**) was synthesized through a 5-step reaction route. Reaction of (**D5**) with three different substituted benzaldehydes viz., 2-hydroxy-benzaldehyde, 2-methoxybenzaldehyde and 2-hydroxy-3-methoxy-benzaldehyde in ethanol afforded Schiff bases (**D6-8**). Yields were fair to moderate (55-70 %). The purity of the compounds was monitored by TLC. These ligands were treated with Cu(II), Co(II) and Ni(II) salts to yield the corresponding complexes.

All the newly synthesized the new ligands (**D6-D8**) and their metal complexes were screened in vivo to evaluate their antibacterial and antifungal efficiencies. These compounds were tested for their antibacterial and antifungal efficiencies are lies in table 1.

The IR spectrum of the (**D6**) exhibits a new squeaky band at 1642 cm^{-1}, attributed to HC=N imine group, which is not present in its precursors, which indicate formation the ligand.[1,2] In the IR spectra of the complexes, this band has shifted to lower side at about 1634 -1637 cm^{-1}. The IR spectrum of ligand exhibited a band at 3506 cm^{-1} due to υ(OH), this band has disappeared in the spectra of complexes.[3] This supports the prpoposition that the ligand coordinate to the metal ions through the nitrogen of υ(C=N) group and oxygen in all the complexes.[4]

The IR spectrum of the ligand (**D7**), showed a new band at 1648 cm^{-1}, attributed to HC=N imine group, not present in its precursors, which indicate formation the ligand.[5] While in IR spectra of the complexes this band has shifted to a lower frequency of about 1634 -1637 cm^{-1}. In the IR spectrum of ligand exhibited band at 1250 cm^{-1} due to υ(C-O), while in complexes spectra this absorption band was shifted at lower in the range (1244-1248) cm^{-1}, indicating that the nitrogen of the amino group and oxygen of OCH_3 group participate in metal coordination.[6] The IR spectrum of the ligand (**D8**), showed the new characteristic band at 1648 cm^{-1}, attributed to HC=N imine group, not present in its precursors, which indicate formation the ligand.[8]

However, in IR spectra of the complexes this band has shifted to a lower vibration in the region of 1634 -1637 cm^{-1}. The spectrum also displayed a band at 1258 cm^{-1} due to υ(C-O), while in complexes spectra this absorption band was shifted to a lower range of 1242-1252 cm^{-1}, suggesting that the nitrogen of the amino group and oxygen of OCH_3 group participate in the metal coordination. Finally, the IR spectra of all the complexes, exhibited new bands at the regions of 437-472 and 522-562 cm^{-1}, which could be assigned to the ν(M-O) and ν(M-N) stretching vibration modes respectively.[9]

The UV spectrum of complex (**S1**) showed a peak at 274 nm which has been assigned to (π → π*) electronic transition. The peaks at 425 nm 873 nm and 962 nm can be assigned to spin-allowed (d-d) electronic transitions of type ($^4A_2 \rightarrow {}^4T_{1(P)}$), ($^4A_2 \rightarrow {}^4T_{1(f)}$) and ($^4A_2 \rightarrow {}^4T_{2(f)}$) respectively. All (d-d) electronic transitions for complex Co(II) suggest a tetrahedral structure around the central metal ion.[10]

The UV spectrum of the complex (**S2**) showed a peak at 279 nm assigned to (π → π*) electronic transition. The peaks at 364 nm 682 nm and 981 nm which can be assigned to spin-allowed (d-d) electronic transition type ($^3T_1 \rightarrow {}^3T_{1(P)}$), ($^3T_1 \rightarrow {}^3A_2$) and ($^3T_1 \rightarrow {}^3T_{1(f)}$) respectively. All (d-d) electronic transitions for Ni(II) complex suggest a square planar structure around the central metal ion.[11]

The UV spectrum of the complex (**S3**) showed a peak at 271 nm assigned to ($\pi \rightarrow \pi^*$) electronic trnasition. The peak at 758 nm which can be assigned to spin-allowed (d-d) electronic transition type ($^2B_1g \rightarrow {}^2B_2g_{(P)}$), transition. The (d-d) electronic transition for Cu(II) complex suggests a square planar structure around the central metal ion.[12]

The UV spectrum of complex (**S4**) showed peak at 268 nm assigned to ($\pi \rightarrow \pi^*$) electronic transition, while the spectrum showed an intense peak at 345 nm, which can be due to ($n \rightarrow \pi^*$) electronic transition for intra-ligand. The peaks at 687 nm and 722 nm can be assigned to spin-allowed (d-d) electronic transition type ($^4T_1g_{(f)} \rightarrow {}^4A_2g_{(f)}$) and ($^4T_1g_{(f)} \rightarrow {}^4T_2g$) transitions respectively. All (d-d) electronic transitions for complex Co(II) suggest an octahedral structure around the central metal ion.[12]

The UV spectrum of complex (**S5**) showed an intense peak at 262 nm, assigned to ($\pi \rightarrow \pi^*$) electronic transition for intra-ligand, while another intense peak at 328 nm may well be due to ($n \rightarrow \pi^*$) electronic transition for intra-ligand. The peaks at 682 nm may be assigned to spin-allowed (d-d) electronic transition type ($^3A_2g \rightarrow {}^3T_1g_{(f)}$), suggesting an octahedral geometry around Ni(II).[14]

The UV spectrum of complex (**S6**) showed an intense peak at 266 nm, assigned to ($\pi \rightarrow \pi^*$) electronic transition for intra-ligand, while the intense peak at 372 nm can be due to ($n \rightarrow \pi^*$) electronic transition for intra-ligand. The peak at 478 nm may relate to spin-allowed (d-d) electronic transition type ($^2Eg \rightarrow {}^2T_2g$) transitions, suggesting a distorted octahedral around Cu(II) metal.[15]

The UV spectrum of complex (**S7**) showed a peak at 249 nm, assigned to ($\pi \rightarrow \pi^*$) electronic transition, while the intense peak at 347 nm can be due to ($n \rightarrow \pi^*$) electronic transition for intra-ligand. The peaks at 522 nm, 669 nm and 786 nm can be assigned to spin-allowed (d-d) electronic transition type ($^4T_1g \rightarrow {}^4T_1g_{(P)}$), ($^4T_1g \rightarrow {}^4A_2g$) and ($^4T_1g \rightarrow {}^4T_2g$) transitions respectively. All (d-d) electronic transitions for complex Co(II) suggest an octahedral structure around the central metal ion.[16]

The UV spectrum of complex (**S8**) showed an intense peak at 243 nm assignable to ($\pi \rightarrow \pi^*$) electronic transition for intra-ligand, while intense peak at 363 nm can be due to ($n \rightarrow \pi^*$) electronic transition for intra-ligand. The peaks at 522 nm and 897 nm may be assigned to spin-allowed (d-d) electronic transition type ($^3A_2g \rightarrow {}^3T_1g_{(P)}$), ($^3A_2g \rightarrow {}^3T_1g$) and ($^3A_2g \rightarrow {}^3T_2g$) transitions respectively suggesting an octahedral geometry around Ni(II).[17]

The UV spectrum of complex (**S9**) showed an intense peak at 247 nm assignable to ($\pi \rightarrow \pi^*$) electronic transition for intra-ligand, while the intense peak at 358 nm may be due to ($n \rightarrow \pi^*$) electronic transition for intra-ligand. The peaks at 486 nm and 576 nm refer to spin-allowed (d-d) electronic transition type ($^2B_1g \rightarrow {}^2Eg$) and ($^2B_1g \rightarrow {}^2A_2g$) transitions, suggesting a distorted octahedral around Cu(II) metal.[18]

Conductivity and magnetic moment measurements

The molar conductance of the complexes (S1- S9) was measured in ethanolic solutions. The conductances of all the complexes were in the range 12.3-l9.2 S cm^2 $mole^{-1}$, indicating the non-electrolytic nature of the complexes.

The magnetic moment values are found to be 2.87and 1.87 BM, for (**S2**) and (**S3**) complexes, respectively, suggesting a square planar geometry, while 5.68 BM, for (**S1**), suggests a tetrahedral geometry. The values for (**S4**-**S9**) complexes are 5.36, 2.87, 1.84, 5.27, 2.91 and 1.83 BM, respectively, suggesting an octahedral geometry for these complexes.

^{1}H NMR spectra

^{1}H NMR spectrum of the Schiff base (**D5**) (Figure 8) showed a singlet at 2.50 ppm for 6 protons for DMSO, a singlet at 3.63 ppm for two protons of CH_2 group and a board signal at 4.63 ppm for two protons of NH_2 group. Also, signals in range 6.63-7.54 ppm that may be assigned to the 13 aromatic protons of phenyl rings. Finally, one proton of NH of indole ring gives a singlet at 9.12 ppm and a singlet signal at 10.01 ppm for one proton of NH group.[19]

^{1}H NMR spectrum of Schiff base (**D6**) (Figure 9) showed a singlet at 2.52 ppm for 6 protons for DMSO, a singlet at 3.35 ppm for two protons of CH_2 group, signals in the range of 6.93-7.54 ppm that might have resulted due to the thirteen aromatic protons of phenyl rings. Also, the spectrum shows a singlet at 8.06 ppm for one proton of the CH=N group. The one proton at NH of indole ring gives as a a singlet at 10.27 ppm and a singlet at 10.53 ppm for one proton of NH group. Finally, a singlet at 1l.17 ppm for one proton of OH group.[20]

^{1}H NMR spectrum of Schiff base (**D7**), (Figure 10) presented singlet signal at 2.50 ppm for 6 protons for DMSO, a singlet at 3 69 ppm for three protons of OCH_3 group, a singlet at 3.94 ppm for two protons of CH_2 group, signals in region of 6.40-7.57 ppm that could be due to the thirteen aromatic protons of phenyl rings. Also, the spectrum shows singlet at 9.32 ppm for one proton of the CH=N group. And one proton at NH of indole ring appears as a singlet at δ 10.32 ppm. Finally, a singlet at 10.82 ppm for one proton of NH group.[20]

^{1}H NMR spectrum of Schiff base (**D8**) (Figure 11) showed singlet at 2.50 ppm for 6 protons for DMSO, a singlet at 3.69 ppm for three protons of OCH_3 group, a singlet at 3.94 ppm for two protons of CH_2 group, signals in range of 6.40-7.57 ppm that may be assigned to the thirteen aromatic protons of phenyl rings. In addition, the spectrum shows a singlet at 9. 32 ppm for one proton of the CH=N group. The one proton of NH of indole ring appears as a singlet at 9.12 ppm and a singlet at of 9.89 ppm for one proton of NH group. Finally, a singlet at of 1l.17 ppm for one proton of OH group.[21]

Mass spectrum

The mass spectrum (Figure 12) of compound (**D4**),[22] showed a molecular ion at *m/z* = 306, which is correspond to the molecular weight of the structure suggested for this compound. The spectrum exhibited various peaks assigned to part of indol (*m/z* = 103, 91) and triazole ring at (*m/z* = 247, 191 and 156). This spectrum also showed peaks at *m/z* = 77, 65 and 51 due to aromaticity of the compound.

Antimicrobial screening

The antifungal and antibacterial efficacies of the compounds were studied against the pathogenic organisms by the disc diffusion method. The bacteria screened were as *E. coli*, *S. aurous*, *B. subtitles*, *P. aeruginosin*, and fungi were as *A. niger*, *A. flavours*, *R. stolonifera* and *C. Albicans*.[23]

The synthesized compounds were used in the concentration of 100 µg mL^{-1} using DMSO as a solvent, ? was used as a standard against both organisms.

The test results, presented in the Table 1, suggest that all the complexes exhibit high activity against the tested bacteria, the rest of the compounds were found to be moderate.

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WATER COMPLEXES WITH AMMONIA AND CARBON DIOXIDE IN POTASSIUM BROMIDE MATRIX

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Keywords: Water intermediates, ammonia, carbon dioxide, hydrogen bond, FTIR spectroscopy, DFT calculation.

In this work a new experimental approach to reveal and study the water intermediates with the gas species in condensed phase at ambient temperature by IR spectral technique is presented. It has been established that the complexes between water molecule and ammonia as well as carbon dioxide in KBr matrix can be formed. *Ab initio* calculations in terms of density functional theory (DFT) at B3LYP/6-311++G(2d,2p) level allowed us to conclude that this binding is accompanied by the hydrogen atom transfer from water to partner molecule.

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Introduction

The hydration of ammonia and carbon dioxide plays a crucial role in many important processes passing in atmosphere and water resources, as well as on the earth surface and in its crust.[1,2] Besides, this phenomenon can be considered as an appropriate model for the nature of the ice formation on the planets and satellites.[3-6] The problem of water association with gases arises also in the producing of high purity materials. [7-11]

The IR spectroscopy is a classical experimental method using for the study of the unstable water clusters.[12,13] However the IR measurements of samples, containing water mixtures is very difficult and similar data have been obtained in the matrixes of noble gases at low temperatures. [14-19] However, most important processes with the participation of a water molecule take place at ambient temperatures.[20]

In earlier work IR technique has been used to detect and study unstable water intermediates at ambient conditions. The fixation of reactive particles in the KBr matrix was taken as a base.[21]

We have modified this method and extended it on the water associates.[22,23] Unlike the neutral matrix material, KBr matter can be an active component supporting the interactions in the system. This feature is provided by the ability of KBr powder to hold water as well as other gas or liquid species.[22,23] Besides this matrix is a very suitable material for IR experiments, because KBr is transparent in a broad IR range.[12]

In this work we have presented the IR spectral manifestation of water intermediates formation with ammonia and carbon dioxide and their transformations in KBr matrix as well as tried to demonstrate the role of potassium bromide in the mechanism of interaction by DFT method.

Experimental and theoretical techniques

Materials

The purity of ammonia and carbon dioxide was no less than 99.999% and was controlled by gas chromatography with a thermal conductivity detector and identification of the main impurities by a conjugate mass selective detector. For this procedure we have used gas chromatograph "Tswet – 800" (Tswet.ru, Russia) with a flow inlet system and in situ analysis as well as chromato-mass spectrometer GCMS - QP2010 Plus (Shimadzu, Japan) with a vacuum sample inlet system of "Valco Instruments Co. Inc." In situ analysis was conducted on a column of 5 m long filled Chromaton N-AW-HMDS (0.16 ÷ 0.20 mm) with 15 % of applied liquid phase E-301. Separation of components was carried out by chromato-mass spectrometry on a capillary column Agilent CP7434 stabilized trifluoropropylmethylpolysiloxane phase at 323 K. Processing of the results was made in "GCMS Real Time Analysis". For the identification of the main impurities the library of mass spectra NIST-11 was used.

Water was deionized (Resistivity 18.2 MΩ•cm at 25°C) by passing through a Millipore Direct-Q system (Millipore, MA, USA).

Samples preparation

The preparation of potassium bromide matrix for the IR study was fully described in our previous papers.[22,23] Briefly, on the first step the KBr powder is saturated by gas (ammonia or carbon dioxide) during two – three hours in a reactor designed for these experiments[23] either together with the water vapour, or by the gas passed through water layer. After that so treated KBr powder is pressed and the obtained pellet is placed in FTIR spectrometer.

FTIR measurements

The ratio of mixed components was chosen in such a way that IR absorption in the selected spectral range with the minimum of background absorption could be recorded. The amount of the KBr powder depends on the fineness of grinding, homogeneity of mixing and the features of pressing system. Therefore, we could not use the universal

ratio of components and have selected the optimal composition for each sample.

Spectra were recorded on a IRAffinity-1 (Shimadzu Co. Inc.) FTIR spectrometer in the range 4000-500 cm^{-1} with a resolution of 2 cm^{-1} and a scan number of 40. All other parameters have corresponded to the ones established by the producer. The accuracy of measurement of wave numbers was controlled by the spectrum of polystyrene, being ±0.2 cm^{-1}.

DFT calculations

Ab initio calculation was carried out in terms of DFT, realized in the computer program GAUSSIAN 09, revision E.01.[24] The geometry, energetic, electronic and spectroscopic characteristics were calculated by the B3LYP functional and the basis set 6-311++G(2d,2p).

Results and discussion

IR study of ammonia-water system

In high frequencies region, a new band at 3240 cm^{-1} was observed (in figure1a, this band is labelled by an asterisk).

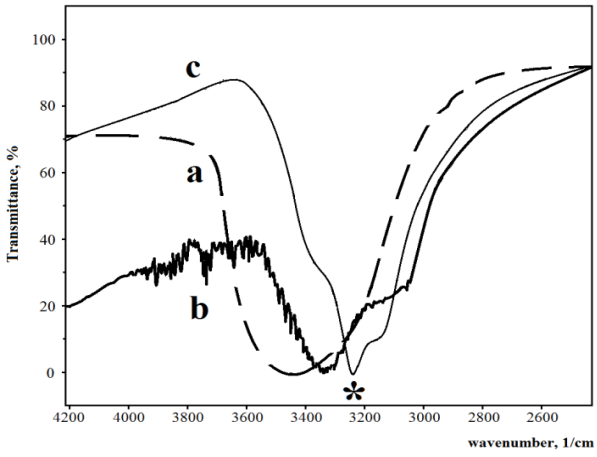

Figure 1a. FTIR spectra of KBr pellets for water-ammonia system saturated by water vapour (a), by ammonia gas (b) and by a mixture of components (c) in a high frequencies region.

For heavy water the band at 2460 cm^{-1} was found (Figure 1b). Since the isotopic shift (ν_H/ν_D = 1.32) agrees well with the expected value, we have assigned these bands to the OH stretching of bonded water in a complex with ammonia molecule.

In the 1800-1200 cm^{-1} region we have seen a new band at 1495 cm^{-1} (Figure 2a), whereas for the heavy water a new band at 1078 cm^{-1} was observed (Figure 2b).

The isotopic shift of mentioned band (ν_H/ν_D =1.39) correlates well with the theoretically predicted value for the XH stretching (X is O or N atom).

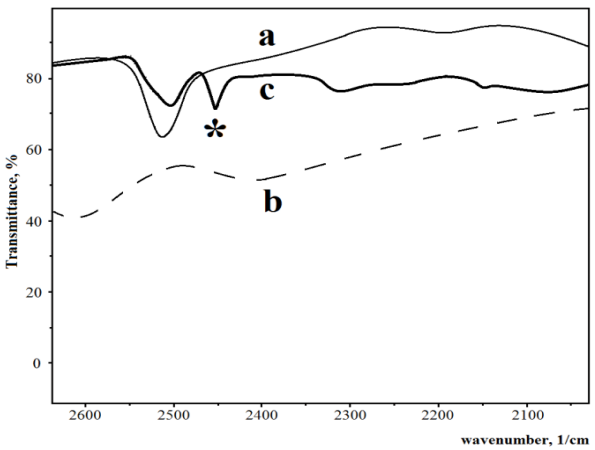

Figure 1b. FTIR spectra of KBr pellets for heavy water-ammonia system saturated by water vapour (a), by ammonia gas (b) and by a mix of components (c) in a high frequencies region.

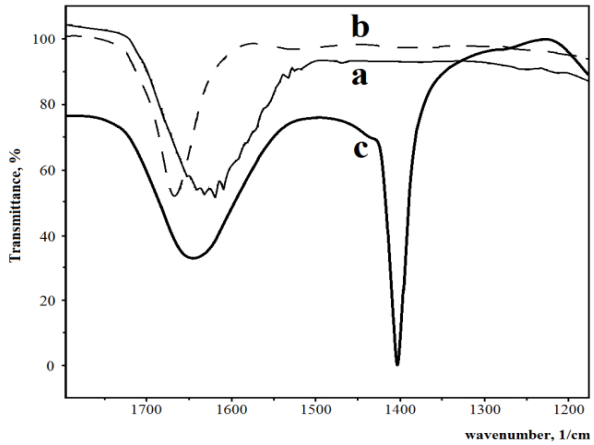

Figure 2a. FTIR spectra of KBr pellets for water-ammonia system in a middle frequencies range saturated by water vapour (a), by ammonia gas (b) and by a mixture of components (c).

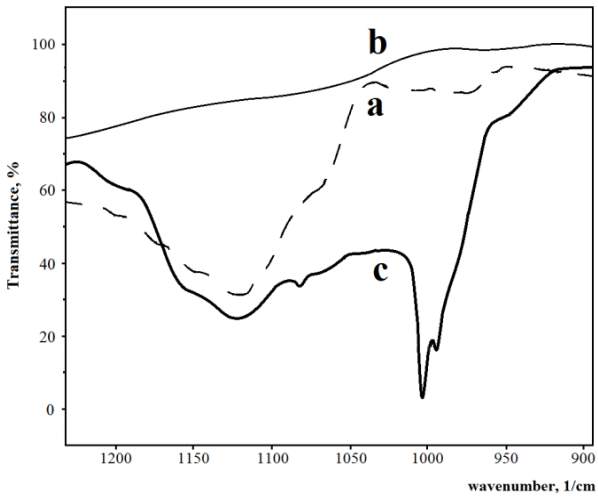

Figure 2b. FTIR spectra of KBr pellets for heavy water-ammonia system in a middle frequencies range saturated by water vapour (a), by ammonia gas (b) and by a mixture of components (c).

The band at 1495 cm^{-1} locates in the same spectral range, where the NH stretching of ammonium salts locates.[12] Therefore, this band can be assigned to the stretching of N-H bond in a water cluster, appearing because of the

hydrogen atom transfer from water to ammonia molecule and formation of $(NH_4)^+$ fragment.

IR Study of Carbon Dioxide-Water System

In the case of carbon dioxide-water system in OH stretching region two new bands at 3120 and 2925 cm^{-1} (in Figure 3a, these are labelled by an asterisks) were observed.

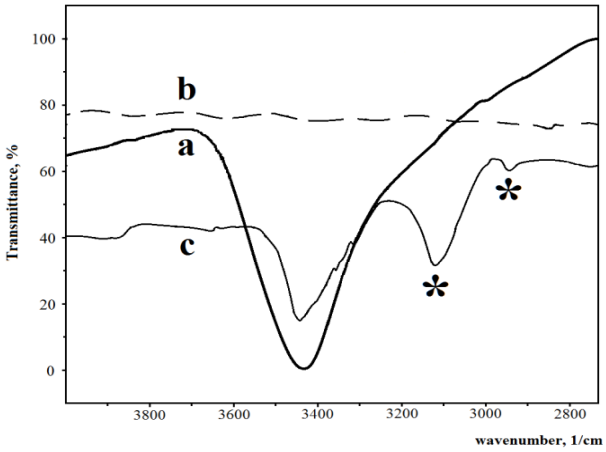

Figure 3a. FTIR spectra of KBr pellets for water-carbon dioxide system in a high frequencies range saturated by water vapour (a), by carbon dioxide gas (b) and by a mixture of thecomponents (c).

For the mixture of carbon dioxide with the heavy water a new band at 2455 cm^{-1} was found (in Figure 3b, it is labelled by an asterisk). Therefore, the mentioned bands correspond to water OH stretching in intermediate with carbon dioxide.

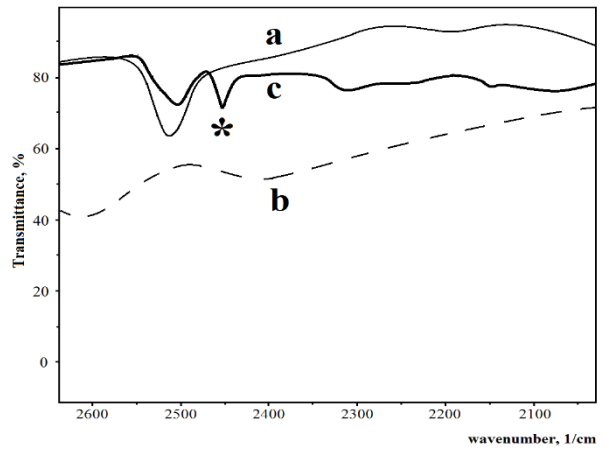

Figure 3b. The FTIR spectra of KBr pellets for heavy water-carbon dioxide system in a high frequencies range saturated by water vapour (a), by carbon dioxide gas (b) and by a mix of components (c).

Besides in the 2600-2000 cm^{-1} region two new bands at 2140 and 2108 cm^{-1} were found (in figure 3b, these are labelled by asterisks). For sample containing heavy water there is no sufficient shift and can be assigned to CO stretching in complex.

The carbon dioxide can form two shapes of water intermediates with O (water)-C (carbon dioxide) and H (water)-O (carbon dioxide) binding.[15] Therefore, the appearance of two new bands in high frequencies region may be relate to the existence of both shapes of water-carbon dioxide intermediates.

In the middle IR region two overlapping bands at 1395 and 1380 cm^{-1} were revealed. These bands don't have isotopic shift for heavy water mixture (Figure 4). If to account that the stretching vibrations of carbonates locate in this range,[12] the mentioned bands can be assigned to CO stretching of CO_3^{2-} anion, arising owing to the transformation of initial water complex in the stable structure (H_2CO_3).

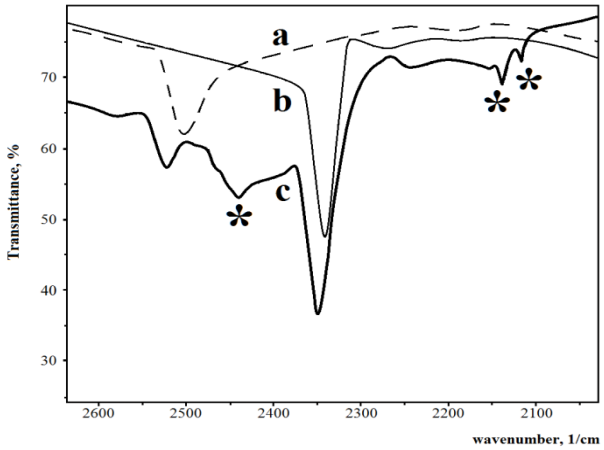

Figure 4. FTIR spectra of KBr pellets for water-carbon dioxide system in a middle frequencies range saturated by water vapour (a), by carbon dioxide gas (b) and by a mixture of the components (c).

DFT study of ammonia-water system

For this system, the *ab initio* calculation predicts the optimized geometry of complex with one water molecule. For the other variants of a cluster structure the set of calculated frequencies contained the negative values and therefore were excluded from the consideration. This geometry is shown in figure 5. The distances KBr-H_2O = 2.59Å and KBr-NH_3 = 2.63Å are considerably longer than the distance NH_3-H_2O = 1.74Å. The calculation gives the frequency of OH-stretching about 3030 cm^{-1}. The big shift of this mode relatively free water OH stretching is caused by the strong interaction between H-atom of water and N-atom of ammonia molecule in the complex.

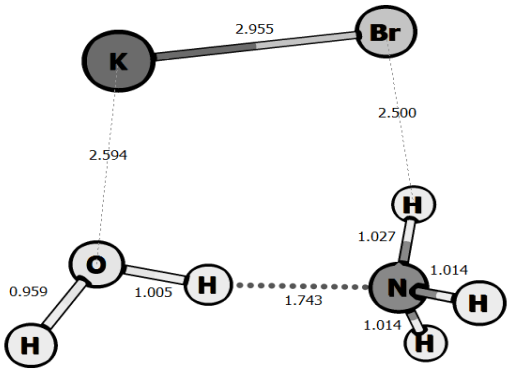

Figure 5. Calculated optimized geometry of the (H_2O/NH_3/KBr) system.

Therefore this interaction can lead to the hydrogen atom transfer from water to ammonia. The computation shows that the transfer about 0.05 Å of water H-atom to NH_3 fragment in complex has energetic barrier less than 8 kJ/mol. However OH-stretching frequency shifts at this transformation from 3030 up to 1698 cm^{-1}, i.e. to the range, in which the new IR bands were observed.

DFT study of carbon dioxide-water System

In this case the calculation gives another mechanism of complex formation in comparison to ammonia-water system.

The obtained structure of water complex with carbon dioxide includes two water molecules. Unlike the previous system, the water and gas components locate on the different sides from KBr centres (Figure 6).

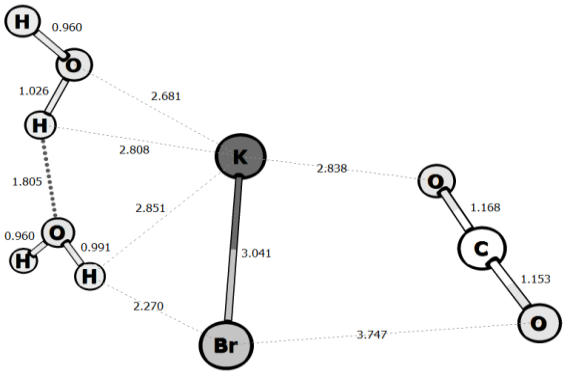

Figure 6. Calculated optimized geometry of the ($2H_2O/CO_2$/KBr) system.

The calculation predicts the hydrogen atom transfer between two water molecules in this cluster. Therefore, it is reasonable to assume that the oxygen atom of carbon dioxide on the first step is protonated by H^+ of the hydronium ion leading to the formation of $(CO_2H)^+$ fragment. Then the OH^- group from water cluster transfers to $(CO_2H)^+$ and as a result the $H_2CO_3 \cdot H_2O$ complex is formed.

On a whole, the DFT data agree with the effects observed in the IR spectra. The appearance of new bands is the manifestation of water binding with ammonia or carbon dioxide molecules accompanied by the hydrogen atom transfer.

Conclusions

The presented data demonstrate that the suggested method using the KBr matrix technique gives the opportunity to investigate the unstable water intermediates at ambient conditions.

The KBr matrix is not neutral in formation of water complexes. Its role is to hold the components as well as to facilitate the molecular transformation.

Acknowledgements

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COMPOSITION CHANGES DURING RE-REFINEMENT OF USED LUBRICATING OILS USING FABRICATED PACKED-BED REACTOR

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Keywords: GC-MS, IR, lubricating oil, PBR, PAHs, re-refined.

This study aimed at developing a laboratory scale packed bed reactor (PBR) and evaluating its performance in re-refining of used lubricating oils. Used and unused motor oil samples of two brands (Mobil Super SAE 20W-50 and Total Quartz 5000 SAE 20W-50) were used in this study. The sorbent materials were a blend of diatomaceous materials in the same mass ratio. The compound types of the oils eluted from the PBR and those of used and virgin oil samples were studied for comparison. FTIR spectroscopy was employed to observe the variations in the types and nature of functional groups present in the various oils as well as to estimate the oxidation products of the oils using peak area increase (PAI). The constituent organic compounds were also identified and quantified using GC-MS. The results revealed that some of the compounds exist in different isomeric forms in the oil samples. The similarity in the classes of compounds is affirmed by their very similar FTIR spectra. GC-MS results indicated that the used oils contained the highest number of compounds, followed by the treated oils and also indicated similar treatment effects on the brands of lubricating oil. The study concluded that the developed reactor is a viable and sustainable technique for re-refining of used lubricating oils.

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Introduction

Motor oil used in automobile engines picks up a number of debris from engine wear. These include metals, sulphur, water, dirt, combustion products, such as ash, and carbon residue. Due to the presence of degraded additives and the aforementioned contaminants, used motor oil disposal can be more environmentally degrading than crude oil pollution. The amount of lubricating oils annually generated worldwide is enormous and is increasing due to increase in automobiles. The large amount of waste engine oils has significant socio-economic and environmental impacts on the society. If discharged onto the land, water or even burnt as a low grade fuel, it may cause serious environmental pollution because harmful metals and other persistent organic and inorganic pollutants are released into the environment. [1] These additives and contaminants may cause both short and long-term adverse effects. Since motor oil does not degrade, used motor oil as the potential to be recycled safely and productively, saving energy and circumventing environmental pollution. [2,3,4]

The conventional approaches of recycling used motor oil either entail a high-cost technology such as vacuum distillation or the use of toxic materials such as sulphuric acid, contaminating by-products with high sulphur concentrations may be made. Blend of vacuum distillation and hydrogenation systems had been used for recycling used engine oil.[5] This approach gives high quality product including high yield. It also removes most of the contaminants from the used oil viz., sulphur, nitrogen and oxygenated compounds as well as enhanced the colour and odour of the oil but it involves high investment cost.[6,7]

Acid-clay technique which had also been severally adopted has disadvantages viz., production of large amount of pollutants, incapability to treat modern multi-grade oils and the difficulty in removing asphaltic impurities.[1,8,9] Solvent extraction method has substituted acid treatment for improving the oxidative constancy and viscosity/temperature characteristics of base oils. The solvent selectively dissolves the unwanted aromatic components, leaving the required saturated components, particularly alkanes, as a distinct phase.[10] Although the oil resulting from this process was akin to that produced by the acid-clay method, it is not cost effective as expensive solvents and vacuum distillation set-ups are essential to carry out the technique.[11,12] In the report of Rincon et al,[13] propane was used as a solvent. Propane can dissolve paraffinic or waxy material and partially dissolve oxygenated material. Asphaltenes which contain heavy condensed aromatic compounds and particulate matter are insoluble in the liquid propane. These properties make propane ideal for recycling the used engine oil, although there are many other concerns that have to be well-thought-out. Propane is expensive and inflammable, so that this procedure is viewed as both cost-ineffective and unsafe. Also, the extraction involves solvent losses, and highly skilled operating maintenance. In addition, extraction occurs at pressures higher than 10 atm and requires high pressure sealing systems which make solvent extraction plants expensive to build and operate. The process also generates significant amounts of hazardous by-products.[9,14]

Another useful method is the membrane technology, here three types of polymeric hollow fibre membranes,

polyethersulphone (PES), polyvinylidene fluoride (PVDF), and polyacrylonitrile (PAN), were used for recycling the used engine oils. The process is carried out at 40 °C and 0.1 MPa pressure.[15] It is an uninterrupted operation procedure, it eliminates metal particles and dusts from used engine oil and also improves the recovered oils liquidity and its flash point. Its shortcomings are that the expensive membranes used in the method may get spoiled or fouled by large particulate matters.[9,15]

The application of the packed-bed reactor using the locally available diatomaceous material is a safe and economical small-scale purification of used lubricating oil since the contaminants have good affinity for diatomaceous materials. This approach is similar to the principle of chromatography separation, hence this study.

Experimental

Materials and their preparation

Unused (virgin) oil samples of two brands (Mobil Super SAE 20W-50 and Total Quartz 5000 SAE 20W-50) were purchased from standard lubricant stations in Ile-Ife, Nigeria, and they were introduced, at the time of routine services, into five selected cars for each brand. The oils were drained after 11 – 12 weeks, and retained as the Used lubricating oil (ULO) samples. The sorbent materials employed consisted of a blend of diatomaceous materials in equal ratio by mass. The sorbent materials were obtained from and prepared at the Federal Institute for Industrial Research, Oshodi (FIIRO), Lagos, Nigeria. Slurry of the sorbent mixture was made and fired at 900°C to produce a cake, which was then ground and screened to produce uniform material of narrow particle-size distribution (1.00-1.50 mm). The particle-size distribution of the ground material was estimated using two sieves of 1.00 and 1.50 mm pore sizes. The ground sorbent was activated by soaking in 6 M H_2SO_4 at 60 °C for 6 h. This was followed by filtration and then drying in a Muffle furnace at about 250 °C for 4 h.

The Packed Bed Reactor consisted of a reservoir which housed the pre-heated used oil, a tap, a reciprocating pump (powered by 1 horse power geared motor), a crank connected to the motor, piston enclosed in a sleeve, pressure gauge, the packed-bed and a collecting vessel. The column was formulated to contain about 10 L quantity of the sorbent while the wall thickness was defined to withstand the required pressure. The packed bed reactor was constructed at the Central Technological Laboratory and Workshop (CTLW), Obafemi Awolowo University, Ile-Ife, Nigeria. The fabricated packed bed reactor is shown in Supplementary material.

The used oil samples were allowed to stand still for two weeks to give way for sedimentation. The top part of each was decanted into stainless pan and heated in open air at about 115 °C for two hours to drive out trapped moisture and some volatile constituents and to reduce its viscosity so that flowing through the packed bed can be enhanced. The hot oil was filtered into the reservoir after which the tap was opened. Five sets of used oil samples from each of the two brands were run through the packed bed reactor (PBR). The constituent organic compounds in the eluted oils from the PBR (treated oils), used and virgin oils were identified and quantified using FTIR spectroscopy and GC-MS. The FT-IR analysis was done at Redeemer University, Ede, Nigeria, while the GC-MS analysis was carried out at University of Ilorin, Ilorin, Nigeria.

Results and discussion virgin

The identification of compounds found in the virgin, treated and used oils were performed by IR and GC-MS methods. Tables 1-9 show the wave numbers as revealed by the FTIR spectroscopy, the corresponding type of vibration, associated bond(s) and functional groups of the oil samples; while their spectra samples are presented in Supplementary materials.

The spectra as well as the peak areas of the various oil samples were very similar. Thus the peak area increase (PAI) values between the used and the treated oils might be very small or the same and consequently, could not be meaningfully determined.[16] Thus, the PAI could not be meaningfully used to estimate the oxidation products.

Table 1. Results of the FTIR analysis of the Virgin Mobil oil sample.

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
655.82	Stretch	C-X	Alkyl halide
721.40	In plane bending (rocking) for C-H of long CH_2	C-H	Alkane
813.99 854.49	Out of plane bending	C-H	Alkene or Aromatic
974.08	Out of plane bending	C-H	Aromatic
1030.02 1170.83	Stretch	C-O	Carboxylic acid
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02 1606.76	Stretch	C=C	Aromatic
1708.99 1732.13 1766.85	Stretch	C=O	Carbonyl
2351.30	Stretch	C≡C	Alkyne
2679.21 2727.44	Stretch	C-H of H-C=O	Aldehyde
2854.74 2922.25	Stretch	C-H	Alkane
3182.65	Stretch	C-H	Alkene Aromatic
3427.62	Stretch	O-H N-H	Alcohol Amine

Table 2. FT-IR analysis of the Virgin Total oil sample.

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
653.89	Stretch	C-X	Alkyl halide
721.40	Bending in plane (Rocking) for C-H of long CH_2	C-H	Alkane
813.99	Out of plane bending	C-H	Alkene or Aromatic
972.16	Out of plane bending	C-H	Aromatic
1032.02 1089.82 1168.90	Stretch	C-O	Carboxylic acid
1230.63 1305.85	Bend (wagging)	CH_2-X	Alkyl halide
1377.22	Out of plane bending	C-H	CH_3
1464.02 1606.76	Stretch	C=C	Aromatic
1705.13	Stretch	C=O	Carbonyl
2359.02	Stretch	C≡C	Alkyne
2673.43 2727.44	Stretch	C-H of H-C=O	Aldehyde
2854.74 2924.18	Stretch	C-H	Alkane
3176.87	Stretch	C-H	Alkene Aromatic
3419.90	Stretch	O-H N-H	Alcohol Amine

Table 3. FT-IR analysis of the Treated Mobil oil sample 1.

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
655.82	Stretch	C-X	Alkyl halide
721.40	In plane bending (Rocking) for C-H of long CH_2	C-H	Alkane
813.99 854.49	Out of plane bending	C-H	Alkene or Aromatic
1030.02 1170.83	Stretch	C-O	Carboxylic acid
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02 1606.76	Stretch	C=C	Aromatic
1707.06 1772.64	Stretch	C=O	Carbonyl
2360.95	Stretch	C≡C	Alkyne
2681.14 2727.44	Stretch	C-H of H-C=O	Aldehyde
2854.74 2926.11	Stretch	C-H	Alkane
3446.91	Stretch	O-H N-H	Alcohol Amine

Table 4. FT-IR analysis of the Treated Mobil oil sample 2.

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
655.82	Stretch	C-X	Alkyl halide
721.40	In plane bending (Rocking) for C-H of long CH_2	C-H	Alkane
813.99 854.49	Out of plane bending	C-H	Alkene or Aromatic
974.08	Out of plane bending	C-H	Aromatic
1030.02 1170.83	Stretch	C-O	Carboxylic acid
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02 1606.76	Stretch	C=C	Aromatic
1707.06 1734.06 1772.64	Stretch	C=O	Carbonyl
2360.95	Stretch	C≡C	Alkyne
2681.14 2727.44	Stretch	C-H of H-C=O	Aldehyde
2854.74 2926.11	Stretch	C-H	Alkane
3184.58	Stretch	C-H	Alkene
3446.91	Stretch	O-H N-H	Alcohol Amine

The organic compounds present in the oil samples with their percentage compositions as elucidated by the GC-MS analysis are presented in Tables 10 and 11, while their chromatograms are displayed in supplementary materials.

The results showed that the used oil samples were more complex in compounds compositions than those of the treated or virgin oil samples. In the used oil samples number of identified compounds ranges from 56 to 98. In the treated samples the number of identified compounds is between 34 and 56 while in the two virgin oil samples the number of identified compounds are 23 and 26. The results, however, do not show clear-cut trend in the compositions as the samples contained similar classes and types of compounds.

Some of the compounds existed in different isomers in the oil samples. A good number of the compounds such as 1-hexacosene, 1,4-oxathiane, 1-chlorooctadecane, 4,5-dihydro-5-methyl-1H-pyrazole, octamethyl-cyclotetrasiloxane, 1,4,5-oxadithiepane, (E)-3-octene, 1-Pentadecene, 1-hexadecene, 1-heptadecene, 3-methyl-1-Pentanethiol, tridecane, thiopropionamide, n-pentadecanol, 2-methyl-1-hexadecanol, t-hexadecanethiol, thiacyclopentane-3-ol, 3-methyl-3-cyclohexen-1-ol, thiacyclopentane-3-ol and 2-ethyl-1-hexanol present in the virgin oils were not detected in the treated or used oils. This may be attributed to their

degradation or isomerisation during use. On the other hand, the numerous compounds in the used oil may be due to contamination by foreign materials, engine parts wearing and degradation as well as isomerisation of the engine oil components during use.

Table 5. FT-IR analysis of the treated total oil sample 1.

Wavenumber. cm^{-1}	Type of vibration	Bond	Functional group
655.82	Stretch	C-X	Alkyl halide
721.40	Bending in plane (Rocking) for C-H of long CH_2	C-H	Alkane
813.99 854.49	Out of plane bending	C-H	Alkene or Aromatic
974.08	Out of plane bending	C-H	Aromatic
1030.02 1170.83	Stretch	C-O	Carboxylic acid
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85 1367.58	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02 1606.76	Stretch	C=C	Aromatic
1705.13	Stretch	C=O	Carbonyl
1737.92	Stretch	C=O	Carbonyl
1770.71	Stretch	C=O	Carbonyl
2031.11	Stretch	C≡C	Alkyne
2681.14 2727.44	Stretch	C-H of H-C=O	Aldehyde
2854.74 2922.25	Stretch	C-H	Alkane
3182.65	Stretch	C-H	Alkene Aromatic
3419.90	Stretch	O-H N-H	Alcohol Amine

With respect to the specificity of the compounds, the most notable is the actual and proportions of polycyclic aromatic hydrocarbons (PAHs). The used oil samples contained appreciably higher number of PAHs, with varying proportions, than the treated oil samples. No PAH was detected in the virgin oil samples. This agreed with the findings of Dominguez-Rosado and Pichtel[17] who reported that the PAHs contents of used motor oils were often between 34 and 90 times higher than new oil.

The identified PAHs and the range of their percentage compositions in the used and treated oil samples respectively are: azulene (Mobil: 0.63-2.64, 1.27-2.05; Total: 0.45-1.19, 1.11-2.62), naphthalene (Mobil: 2.61- 2.65, 0.94-2.54; Total: 3.20-3.28; 2.34-2.36), 1-methylnaphthalene (Mobil: 1.52-2.14, 1.91-1.92; Total: 0.35- 0.41, 0.89-1.65), 2-methylnaphthalene (Mobil: 0.42-1.58, 0.70-1.44; Total: 0.36-1.48, 1.12-1.59), 1,3-dimethylnaphthalene (Mobil: 0.34-0.35, ND; Total: ND, ND), 1,5-dimethylnaphthalene (Mobil: ND, ND; Total:0.34-0.38, ND), 1,6-dimethylnaphthalene (Mobil: ND, ND; Total: 0.37-1.46,ND), 1,7-dimethylnaphthalene (Mobil: 0.31-0.82, ND; Total: 1.33-1.34; Total: ND, ND), 2,7-dimethylnaphthalene (Mobil: ND,ND; Total: 0.33-1.18, ND), 2-ethylnaphthalene (Mobil: ND, ND; Total: 0.61-0.63, ND), 1,4,6-Trimethylnaphthalene (Mobil: ND, ND; Total: 0.68-0.69, ND), 2,3,6-Trimethylnaphthalene (Mobil: ND, ND; Total: 0.34-0.39, ND), 1,2,3,4-Tetrahydronaphthalene (Mobil: 0.99-1.01, ND; Total: 0.97-0.99, ND), 6-Ethyl-1,2,3,4-Tetrahydronaphthalene (Mobil: ND, ND; Total: 0.20-1.97, ND), 1,2,3,4-

Table 6. FT-IR analysis of the Treated Total oil sample 2.

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
655.82	Stretch	C-X	Alkyl halide
721.40	In plane bending	C-H	Alkane
813.99 854.49	Out of plane bending	C-H	Alkene or Aromatic
974.08	Out of plane bending	C-H	Aromatic
1030.02 1170.83	Stretch	C-O	Carboxylic acid
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02 1604.83	Stretch	C=C	Aromatic
1707.06 1732.13 1772.64	Stretch	C=O	Carbonyl
2360.95	Stretch	C≡C	Alkyne
2681.14 2727.44	Stretch	C-H of H-C=O	Aldehyde
2854.74 2924.18	Stretch	C-H	Alkane
3182.65	Stretch	C-H	=C-H
3443.05	Stretch	O-H N-H	Alcohol Amine

Table 7. FT-IR analysis of the Used Mobil oil sample 1.

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
655.82	Stretch	C-X	Alkyl halide
721.40	In plane bending	C-H	Alkane
813.99 854.49	Out of plane bending	C-H	Alkene or Aromatic
974.08	Out of plane bending	C-H	Aromatic
1030.02 1155.40	Stretch	C-O	CO_2H, CO_2R,--C-OH
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3

1464.02	Stretch	C=C	Aromatic
1606.76			
1705.13	Stretch	C=O	Carbonyl
1732.13			
1770.71			
2038.83	Stretch	C≡C	Alkyne
2173.85			
2359.02			
2679.21	Stretch	C-H of	Aldehyde
2727.44		H-C=O	
2854.74	Stretch	C-H	Alkane
2924.18			
3173.01	Stretch	C-H	=C-H
3431.48	Stretch	O-H	Alcohol
		N-H	Amine

Table 8. FT-IR analysis of the Used Mobil oil sample 2

Wavenumber, cm^{-1}	Type of Vibration	Bond	Functional group
657.75	Stretch	C-X	Alkyl halide
721.40	Bending in plane	C-H	Alkane
813.99	Out of plane	C-H	Alkene or
854.49	bending		Aromatic
972.16	Out of plane bending	C-H	Aromatic
1030.02	Stretch	C-O	Carboxylic
1089.82			acid
1230.63	Bend (wagging)	CH_2-X	Alkyl halide
1305.85	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02	Stretch	C=C	Aromatic
1604.83			
1705.13	Stretch	C=O	Carbonyl
1776.50			
2359.02	Stretch	C≡C	Alkyne
2727.44	Stretch	C-H of	Aldehyde
2854.74		H-C=O	
2924.18	Stretch	C-H	Alkane
3184.58	Stretch	C-H	Alkene Aromatic
3435.34	Stretch	O-H	Alcohol
		N-H	Amine

Tetrahydro-1,8-dimethylnaphthalene (Mobil: 0.31-0.42, ND; Total: 0.21- 0.41, 0.20-0.48), Pyrene (Mobil: 0.26-0.91, 0.22-0.23; Total: 0.25-0.35, ND), Anthracene (Mobil: 0.66-0.92, 0.28-0.30; Total: 0.93-1.02), Phenanathrene (Mobil: 0.25-0.27, ND; Total: 0.32-2.61, ND), 3-Methylanthracene (Mobil: ND,ND; Total: 0.33-0.34, ND), 1,6-Dimethylanthracene (Mobil: 0.37-0.42, ND; Total: ND, ND), 1,6-Dimethylphenanthrene (Mobil: ND, ND; Total: 0.35-0.36, ND), Acenaphthylene (Mobil: 0.39-0.47, ND; Total: 0.17-0.46, ND), Benzo(a)Pyrene (Mobil: 0.29-0.66, ND; Total: 0.30-0.31, ND), 1,2,3,5,8,8a-Hexahydronaphthalene (Mobil: ND, 1.92-1.94; Total: ND, 0.21-1.81), 3,4-Dihydro-3-methyl-1(2H)-naphthalenone (Mobil: ND, ND; Total: 0.09-0.16), 1,5-Dimethoxy-9,10-anthracenedione (Mobil: ND, 1.37-7.96; Total: ND, ND). The presence of PAHs in high concentrations in the used lubricating oils could be as a result of the action of internal combustion engine which subjects the oil to a lot of thermal activities. The high reduction in number and/proportions of the PAHs in the used oil samples as observed in the treated samples is an indication that the sorbent materials have good uptake for these carcinogenic compounds.

Moreover, the lower numbers of various compounds in the treated oil samples than their corresponding used oil samples is an indication that a fraction of the constituent compounds in the used oil sample were removed by the PBR. The higher number of low molecular weight compounds in the used oil samples than the treated or the virgin oil samples showed that the used oil samples contained more volatile compounds or more fragile compounds that can thermally break down more readily.

Table 9. FT-IR Analysis of the Used Total oil sample 1

Wavenumber, cm^{-1}	Type of vibration	Bond	Functional group
659.68	Stretch	C-X	Alkyl halide
721.40	Bending in plane	C-H	Alkane
812.06	Out of plane bending	C-H	Alkene or Aromatic
974.08	Out of plane bending	C-H	Aromatic
1031.95	Stretch	C-O	Carboxylic acid
1155.40			
1230.63	Bend (wagging)	CH_2-	Alkyl halide
1309.71	Rock	C-H	Aromatic
1377.22	Out of plane bending	C-H	CH_3
1464.02, 1604.83	Stretch	C=C	Aromatic
1705.13, 1774.57	Stretch	C=O	Carbonyl
2036.90, 2360.95	Stretch Stretch	C≡ C	Alkyne
2679.21 2727.44	Stretch	C-H of H-	Aldehyde
2854.74, 2924.18, 2955.04	Stretch	C-H	Alkane
3192.30	Stretch	C-H	Alkene Aromatic
3446.91	Stretch	O-H N-H	Alcohol Amine

The difference in organic compounds compositions of the two virgin oil samples may linked to the types of the base oil as well as types of additives used by the respective manufacturer in their production.

Although the virgin oil samples contain longer straight alkyl chain groups, which are expected of the base oil of lubricating locomotive engine oils, than the treated and used oil samples, the numbers of such long straight chains are very few.

The virgin oil samples contain more of branched chains than straight chains alkanes; the longest straight alkane chain being tridecane. The longer chains are alkenes [1-Hexadecene, 1-Heptadecene and 1-Hexacocene], alcohols [(Z)-2-(9-octadecenyloxy)ethanol**,** n-Pentadecanol, 2-Methyl-1-hexadecanol], thiol [Tert-hexadecanethiol] and ester [6,9-Octadecadiynoic acid, methyl ester]. Even the alkenes and other identified unsaturated compounds are more common, both in number and proportion, than the alkanes. These observations suggest breaking in chain lengths and/or rearrangements of the components during the gas chromatography analysis. Moreover, the inconsistency in the retention times of a particular component in the various samples is an indication of high variation in the constitutions of the samples and that interactions among the various components in the samples play a role in their relative movement through the capillary.

Table 10. Identified compounds and their percentage compositions in the Mobil oil samples.

Identified Compounds	**% Composition (range)**		
	Used	**Treated**	**Virgin**
Ethylbenzene	2.06-6.32	2.59	
o-Xylene	2.5	3.04-4.48	6.01
m-Xylene	5.01-6.32	1.02-2.29	
p-Xylene	3.13-4.43	2.24-7.14	2.46
n-Propylbenzene	0.98-1.41	0.92	
Isopropylbenzene	0.34-0.41	1.27	
1,4-Oxathiane			6.05
1,2,3-Trimethylbenzene	1.71-2.30	2.31-2.43	
1,2,4-Trimethylbenzene	1.83-5.41	1.32-4.34	
Mesitylene	2.29-8.11	0.96-9.10	
1-Methyl-2-propylbenzene	2.35		
1-Methyl-3-propylbenzene	0.80		
1,2-Diethylbenzene	1.15		
2-Ethenyl-1,4-dimethylbenzene	2.91	0.95-1.01	
2-Ethenyl-1,3,5-trimethylbenzene	0.40		
1-Ethyl-2-methylbenzene	2.11-5.41	2.72-4.52	
1-Ethyl-3-methylbenzene	1.56-7.71	1.86	
1-Ethyl-4-methylbenzene	4.10	1.04-1.42	
1-Ethyl-3,5-dimethylbenzene	1.56-1.10		
2-Ethyl-1,4-dimethybenzene	0.97-0.97	1.04-1.99	
4-Ethyl-1,2-dimethylbenzene	0.97-5.41		
1-Ethyl-2,4,5-trimethylbenzene	0.98-0.97		
1-Methyl-3-propylbenzene	2.34-2.35	0.92	
1-Methyl-4-propylbenzene	0.85-0.86		
1-Methyl-3-isopropylbenzene	0.80		
1-Ethyl-3-isopropylbenzene	0.44		
1-Ethyl-4-isopropylbenzene	0.36-0.53		
1,3-Diethyl-5-methylbenzene	0.30-0.31		
1,3-Dimethyl-5-isopropylbenzene	0.97		
1,2,3,4-Tetramethylbenzene	1.63	1.21	
1,2,3,5-Tetramethylbenzene	0.67-0.94	1.59	
1,2,4,5-Tetramethylbenzene	0.67-1.63		
Pentamethylbenzene	0.44		
2,4-Diethyl-1-methylbenzene	0.29-0.56		
1-Methyl-4-sec-butylbenzene	0.66-1.19		
p-Allyltoluene	0.53		
3-(m-Tolyl)-1-butene	0.53-1.07		
3-(p-Tolyl)-1-butene	0.53		
2-Methyl-3-phenyl-2-butene	1.08		
o-Allyltoluene	3.52	0.96	
p-Ethylcumene	0.53		
3,4-Dimethylcumene	0.56-0.57	1.12	
2-Butenylbenzene	0.28-3.52	2.23	
1-(2-Butenyl)-2,3-dimethylbenzene	0.30		
(E)-4-(2-Butenyl)-1,2-dimethylbenzene	0.36		
2-Methylstyrene	2.29-3.73		
4-Methylstyrene	3.73-4.10		
2,4-Dimethylstyrene	3.34-4.16	2.21	
2,5-Dimethylstyrene	2.91-4.16	1.84	
2,6-Dimethylstyrene	3.87		
2,4,6-Trimethylstyrene	0.40-2.09		
2-tert-butyltoluene	1.22-1.23		
1,4-Diisopropenylbenzene	0.52	2.14	
5-Ethyl-2-methyldecane	0.36-0.37		
o-Cymene	0.92-1.15	1.06	
m-Cymene	0.80		
p-Cymene	0.29-5.41	1.99-3.89	
2-Phenyl-2-pentene	1.08		
2-Methyl-1-phenyl-2-butene	0.63-0.88		
(2-Bromocyclopropyl)benzene	0.72		
1-Chlorooctadecane			2.44
1,2-Dichlorocyclohexane	0.76		
1,2-Dipentylcyclopropene			2.56
1-Methyl-1-silabenzocyclobutene	2.33		
3,5,5-Trimethylcyclohexene	0.38		
Benzocycloheptatriene	0.46-0.47	0.97-2.11	
Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	2.65	2.13-2.52	
1-Methyl-tricyclo[5.2.1.0(2,6)]dec-4-ene	0.75-0.76		
7-Methylbicyclo[4.2.0]octa-1,3,5-triene	0.49-3.03	0.87-3.14	
7-Ethylbicyclo[4.2.1]nona-2,4,7-triene	0.91-1.51	0.93	
3-Tert-butyl-7,7-dimethyloct-3-ene-1,5-diyne	0.52	1.93	
2-Thiopheneacetyl chloride	0.78-0.96	0.93	
6,6-Dimethylfulvene	0.32-6.67	1.03-8.66	
1,2,3,4-Tetramethylfulvene	1.15-2.33		
1,3,8-p-Menthatriene	4.38-4.39	0.92-2.11	
1,2,3-Trimethylindene	0.52		
1-Chloro-2,3-dihydro-1H-indene	3.49-3.50		
2,3-Dihydro-2,2-dimethylindene	1.53-1.54		
2,3-Dihydro-1,6-dimethyl-1H-Indene	0.55-0.56	1.01-1.30	
2,3-Dihydro-4,7-dimethyl-1H-Indene	0.80-1.24		
2,3-Dihydro-5,6-dimethyl-1H-indene	0.53		
2,3-Dihydro-1,1,5-trimethyl-1h-indene	0.36		
Octahydro-2-methylene-4,7-Methano-1H-indene	0.50-0.51		
2,3,6,7-Tetrahydro-3a,6-methano-3aH-indene	3.86-3.87	1.63-1.73	

3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene	1.57-2.34	1.82	
2,3,4,5,6,7-Hexahydro3a,6-Methano-3aH-indene	0.98-0.99		
(Z)-2-(9-Octadecenyloxy)ethanol			2.76
(Z)-4-Nonen-2-yne	2.34		
(Z)-3-Dodecene			3.94
(Z)-2-Methyl-4-Decene	1.43-1.44		
Trans-cinnamyl bromide	0.72		
Decane	0.30-0.31		
Dodecane	0.66		2.66
Tetradecane	0.46-0.47		
1-Dodecene	0.86-0.90	0.93-0.94	
4-Methyl-2H-benzopyrane	1.04		
4,5-Dihydro-5-methyl-1H-pyrazole			2.76
1,4,5-Oxadithiepane			2.69
5,6,7,8-Tetrahydroquinoxaline	0.50		
4-Nitropyridine-1-oxide			2.01
1-Heptadecene			3.97
1-(Trimethylsilyl)-1-propyne			4.07
2-(1-Hydroxyethyl)norbornadiene	2.34		
1-Hexadecene			4.09
1-Hexacosene			2.88
Deltacyclene	0.72		
2-(p-Tolyl)ethylamine	0.52-0.53		
N-2-Dimethyl-N-nitro-1-Propanamine	1.24-2.33		
N,N-Di(trichloroacetyl)-2-Phenylethylamine	0.37		
2,3,4-Trimethylbenzeneethanamine	0.31		
Octamethylcyclotetrasiloxane			4.45
2,2'-Thiobisbutane	0.29-0.31	0.29	
1-Octanethiol	3.21-3.22	2.03-8.41	
2-Ethyl-1-hexanethiol	0.79-0.80	5.48	2.56
4-Methyl-2-Pentanethiol			17.05
1-Dodecanethiol	1.66	1.98	
Tert-hexadecanethiol			3.73
Thiacyclopentane-3-ol			3.93
3-Methyl-3-cyclohexen-1-ol			2.01
2-Ethyl-1-hexanol			12.48
5-Methyl-1-heptanol	0.95-0.96		
1-Octanol	6.45	6.45	
2-Butyl-1-octanol	0.33-0.34		
n-Pentadecanol			1.81
2-Methyl-1-hexadecanol			1.98
p-Isopropenylphenol	1.58-1.59		
3,3,4-Trimethyl-4-p-tolylcyclopentanol	0.49-0.50		
α-Methyl- β-nitrobenzenepropanol	0.98-0.99		
Bicyclo[2.2.1]heptane-2-methanol	0.61		
9-Methyltricyclo[4.2.1.1(2,5)] deca-3,7-diene-9,10-diol	0.39-0.40		
17-Pentatriacontene	3.05-4.48	3.43-4.92	
Biphenylene	0.47		
Azulene	0.63-2.64	1.27-2.05	
Naphthalene	2.61-2.65	0.94-2.54	
1-Methylnaphthalene	1.52-2.14	1.91	
2-Methylnaphthalene	0.42-1.58	0.70-1.44	
1,3-Dimethylnaphthalene	0.34-0.35		
1,7-Dimethylnaphthalene	0.31-0.82		
1,2,3,4-Tetrahydronaphthalene	0.99-1.01		
1,2,3,4-Tetrahydro-1,8-dimethylnaphthalene	0.31-0.42		
Pyrene	0.26-0.91	0.22	
Anthracene	0.66-0.92	0.28	
Phenanathrene	0.25		
1,6-Dimethylanthracene	0.37		
Acenaphthylene	0.39-0.47		
Benzo(a)Pyrene	0.29-0.66		
Isopropyl-2-thiopheneacetate			2.68
Octylchloroformate	0.35-0.36		
Pentachloropropionic acid octyl ester	8.28-8.29		
Methyl 6,8-octadecadiynoate	0.63-0.65		
12,15-Octadecadiynoic acid, methyl ester	0.26 0.27		
10,12-Tricosadiynoic acid, methyl ester	0.41-0.47		
5,8,11-Heptadecatriynoic acid, methyl ester	0.62-0.66		
5,8,11-Eicosatriynoic acid, methyl ester	0.32-0.39		
3-Hydroxydodecanoic acid	2.00-2.01	4.86	
3,7,12-Trihydroxycholestan-26-oic acid	0.37	7.73-9.21	
3,7-Dimethyl-6,7-di(methylthio)octanal	0.80-1.63	1.72	
2-Ethylcyclopentanone	0.57-0.58		
1-(4-Isopropenyl) phenylethanone	0.40		
1-(4-Methylphenyl)ethanone	5.41		
Pentamethylbenzenesulphonamide	1.03-1.04		
2-(2-Methyl-5-nitro-imidazol-1-yl)-N-phenethyl-acetamide	0.37		
Barbital	0.36-0.82		
Amobarbital	0.26-0.46		

Table 11. Identified compounds and their percentage compositions in the Total oil samples.

Identified Compounds	% Composition (Range)		
	Used	Treated	Virgin
Ethylbenzene	1.40-2.31	2.08-2.39	
o-Xylene	0.24-2.28	3.89	1.44
m-Xylene	2.26-5.93	0.95-0.96	1.13
p-Xylene	1.81-5.87	2.94-6.14	1.69
n-Propylbenzene	0.60-1.42	0.98-0.99	
Isopropylbenzene	0.34-0.35	5.06	

1,2,3-Trimethylbenzene	1.36-2.26	1.72-2.33
1,2,4-Trimethylbenzene	2.06-4.53	3.44-8.53
Mesitylene	1.17-2.22	1.82-7.91
1-Ethyl-2-methylbenzene	0.81-2.09	1.32, 1.57
1-Ethyl-3-methylbenzene	3.00-6.74	1.41-4.48
1-Ethyl-4-methylbenzene	0.23	4.52
1-Ethyl-2,4-dimethylbenzene	1.03-1.04	0.67
1-Ethyl-3,5-dimethylbenzene	1.18	
2-Ethyl-1,3-dimethylbenzene	1.41	
2-Ethyl-1,4-dimethybenzene	0.97-3.50	1.11-3.38
4-Ethyl-1,2-dimethylbenzene	5.18	0.93-0.95
1-Methyl-2-propylbenzene	2.37	0.92
1-Methyl-3-propylbenzene	0.82-2.33	0.89-0.92
1-Methyl-3-isopropylbenzene	0.85-0.99	
4-Butyl-1-methylbenzene	0.21	1.14-1.20
1-Ethyl-4-isopropylbenzene	0.79	
1-Ethyl-2,4,5-trimethylbenzene	1.39	0.54
1,2,3,5-Tetramethylbenzene	1.20	
1,2,4,5-Tetramethylbenzene	0.67-1.64	1.31─1.34
Pentamethylbenzene	0.43-0.83	
1,3-Diethyl-5-methylbenzene	0.31-0.32	
1,4-Diethyl-2-methylbenzene	0.44-0.80	0.46-1.83
1,3-Dimethyl-5-isopropylbenzene	0.30-0.96	
2,4-Diethyl-1-methylbenzene	0.96-0.97	
1-Methyl-4-sec-butylbenzene	1.16-1.17	
3-(m-Tolyl)-1-butene	0.63	1.02-1.08
1,4-Dimethyl-2-t-butylbenzene	1.15	
3-(p-Tolyl)-1-butene	0.61-0.62	
2-Methyl-2-phenyl-2-buteene	2.04-2.05	
o-Allyltoluene	3.35-3.36	
1,4-Diethyl-2,5-dimethylbenzene	0.65	
2-Propenylbenzene	0.52	3.66-3.67
2-Butenylbenzene	0.45-1.39	
1-(2-Butenyl)-2,3-dimethylbenzene	0.22	
(E)- 4-(2-Butenyl)-1,2-dimethylbenzene	0.35-0.43	
2-Methylstyrene	3.67-3.68	4.30
2,4-Dimethylstyrene	2.15-4.15	0.97-2.93
2,5-Dimethylstyrene	4.46	
2,4,6-Trimethylstyrene	1.78-2.81	0.40
o-Isopropenyltoluene	1.58	
2-Methyl-2-phenylbenzene	0.43	
1,2-Diisopropylbenzene	1.21	
4-Heptynylbenzene	0.31-0.32	
o-Cymene	1.15-2.59	1.06
p-Cymene	3.16	1.99-4.46
3-Methylphenylacetylene	3.42-3.43	
2-Phenyl-2-pentene	1.03-1.04	
2-Methyl-1-phenyl-2-butene	2.07-4.06	2.44-4.80
2-Isopropenylcumene	0.42-0.42	
3-Isopropenylcumene	0.49-0.50	0.42-0.81
Chloromethyl n-propyl sulphide		6.04
2,4-Dimethylbenzyl chloride	0.17	
1,2-Dichlorocyclohexane	0.75-0.78	1.04-1.06
1-Methyl-1-silabenzocyclobutene	2.28-2.29	
1-Ethynyl-1-Cyclohexene	4.20-4.21	
Benzocycloheptatriene	4.42	1.03-1.85
Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	2.61-3.20	1.71-2.24
1-Methyl-tricyclo[5.2.1.0(2,6)]dec-4-ene	0.6-0.64	
3-Methyl-4-methylenebicyclo[3.2.1]oct-2-ene	0.80	
7-Ethylbicyclo[4.2.1]nona-2,4,7-triene	1.49-2.07	0.36-0.93
7-Isopropylbicyclo[4.2.0]octa-1,3,5-triene	0.52	
1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	0.19	
3-Tert-butyl-7,7-dimethyloct-3-ene-1,5-diyne	0.47-0.48	1.88
2,4-Dimethylheptane	0.16	
2,3-Dimethyl-cyclohexa-1,3-diene	0.23	
1,2,3,4-Tetramethyl-5-methylene-1,3-Cyclopentadiene	2.34	
1,3,8-p-Menthatriene	1.66	1.01-1.02
Indane	2.60	6.56
Indene	1.65-3.46	0.93-0.94

2,3-Dihydro-1,3-dimethyl-1H-indene	1.20		
2,3-Dihydro-1,6-dimethyl-1H-Indene	0.51	0.94-1.30	
2,3-Dihydro-3,3-dimethyl-1H-Inden-1-one	0.17		
2,3-Dihydro-4,7-dimethyl-1H-Indene	1.21-1.22	0.81	
2,3-Dihydro-1,1,3-trimethyl-1H-indene	0.16		
2,3-Dihydro-1,1,5-trimethyl-1H-indene	0.39-1.07		
Octahydro-4,7-methano-1H-indene	0.50	1.32-1.33	
Octahydro-2-methylene-4,7-Methano-1H-indene	0.44	0.52	
2,3,6,7-Tetrahydro-3a,6-methano-3aH-indene	3.85	1.63-2.47	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene	2.20	1.59-1.62	
(Z)-4-Nonen-2-yne	2.29-2.36		
(E)-3-Octene			15.21
(E)-3-Methyl-4-Decene	0.23		
(Z)-2-Methyl-5-undecene	1.11	1.02	2.51
Trans-cinnamyl bromide	0.71-0.73		
3-Methyl-2-Heptene			6.81
Decane	0.31-0.45		
Dodecane	0.34-0.39		1.53
3-Methyldecane	0.24		
2,6-Dimethylundecane	0.40		
2-Bromododecane	0.18		
7-Methyltridecane	0.27		
Tridecane			1.56
1-Dodecene	0.47-0.48	0.98-1.02	
1-Pentadecene			1.23
1-Heptadecene			1.23
2-Tolyloxirane	0.43		
3-Methyl-1H-pyrrole	0.56		
2-(Isopropyl)-1H-benzimidazole	0.33		
2-Isopropyl-1H-pyrrolo[2,3-b]pyridine	0.93		
4-Methyl-2H-benzopyrane	1.00-1.01		
5,6,7,8-Tetrahydroquinoxaline	2.59-3.12		
1,5-Dimethyl-2-pyrrolecarbonitrile	0.62		
Trichlorovinylsilane	0.62		
1,3,6-Trioxocane	0.21-0.79	0.19-1.22	
Hexacosane	0.45		
17-Pentatriacontane	0.66	1.10	
Deltacyclene	0.71		
4-Propylbenzenamine	0.90		

p-Pentylaniline	0.35		
2,3,4-Trimethylbenzeneethanamine	6.58		
N-2-Dimethyl-N-nitro-1-Propanamine	0.46-0.47		
N,N'-Dibenzylideneethylenediamine	1.36		
N,N-Di(trichloroacetyl)-2-Phenylethylamine	0.36-0.37		
2,3,4-Trimethylbenzeneethanamine	6.58-6.58		
2,2'-Thiobisbutane	0.15-0.26	0.29	1.24
1-Octanethiol	1.45-8.34	1.96-8.81	19.12
3-Methyl-1-Pentanethiol			7.42
4-(9-Borabicyclo[3.3.1]non-9-yloxy)-2-thiapentane			1.43
5-Methyl-1-heptanol		0.97	5.51
6-Methyl-1-heptanol			4.93
1-Phenyl-1-cyclopentanol	0.56		
3,3,4-Trimethyl-4-p-tolylcyclopentanol	0.26-0.26		
(Z)-2-(9-Octadecenyloxy)ethanol			1.44
3,9-Dimethyltricyclo[4.2.1.1(2,5)]dec-3-en-9-ol	0.28		
9-Methyltricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-diol	0.41		
Biphenyl	0.32		
3-Methyl-1,1'-biphenyl	0.74		
4-Methyl-1,1'-biphenyl	0.22		
Biphenylene	0.20-0.63		
Azulene	0.45-1.19	1.11-2.62	
Naphthalene	3.20	2.34	
1-Methylnaphthalene	0.35	0.89-1.65	
2-Methylnaphthalene	0.36-1.48	1.12-1.59	
1,5-Dimethylnaphthalene	0.34		
1,6-Dimethylnaphthalene	0.37-1.46		
1,7-Dimethylnaphthalene	1.33-1.35		
2,7-Dimethylnaphthalene	0.33-1.18		
2-Ethylnaphthalene	0.61─0.69		
1,4,6-Trimethylnaphthalene	0.68		
1,6,7-Trimethylnaphthalene	0.43		
2,3,6-Trimethylnaphthalene	0.34-0.39		
6-Ethyl-1,2,3,4-tetrahydronaphthalene	0.20-1.97		
1,2,3,4-Tetrahydronaphthalene	0.98		
1,2,3,4-Tetrahydro-1,8-dimethylnaphthalene	0.21-0.41	0.48	

Pyrene	0.25-0.35		
Anthracene	1.02-1.32		
Phenanathrene	0.32-2.61		
2-Methylphenanthrene	0.33-0.42		
3-Methylanthracene	0.33-0.34		
1,6-Dimethylphenanthrene	0.35-0.36		
Acenaphthylene	0.17-0.46		
Benzo(a)Pyrene	0.30-0.31		
3,4-Dihydro-3-methyl-1(2H)-naphthalenone	0.16		
Thiophene-2-acetic acid, cyclobutyl ester	0.59		
Methyl 6,8-octadecadiynoate	0.60-0.61		
6,9-Octadecadiynoic acid, methyl ester			1.12
4-[(Tetrahydro-2H-pyran-2-yl)oxy]butanal	0.64		
3,7-Dimethyl-6,7-di(methylthio)octanal	0.78-0.79	1.62	
3-(4-Methylphenyl)-2-Propenal	0.98		
1-(4-Methylphenyl)ethanone	5.06-5.06		
5-Methylene-4,5,6,6a-tetrahydro-3ah-pentalen-1-one	0.46 ─0.48		
Thiopropionamide			1.65
Butethal	0.32-0.34		
Barbital	0.35-0.36		
Pentobarbital	0.32-0.81		

Conclusion

The three sets of the oil samples (used, treated and virgin) from each of the two brands contained similar classes of compounds but varying degree in number, specificity and proportion of compounds with the treated oil samples saddled between the other two sets. The study showed that the sorbent materials have good uptake for polycyclic aromatic hydrocarbon, soot and other impurities in used lubricating oil.

The application of the packed bed reactor using the locally produced materials used in this study is recommended for small-scale pre-treatment of used lubricating oil before its subsequent uses. Further improvement on the packed bed reactor system is needed to be pursued with the objective of conducting theoretical studies of this work on the basis of the experimental data. This will enable the designing of the packed bed reactor and development of a dynamic model in order to estimate values of the parameters characterizing the interaction of transport phenomena with diatomaceous sorption in packed bed used lubricating oil re-refining.

The information from the study will help in the development of highly efficient packed bed reactor for the recycling of used lubricating oil by working on the method optimization. Consequently, the packed bed reactor may be enlarged using principles of geometry and dynamic similarity, and possibly modified for low-cost and environmental friendly large-scale used lubricating oil recycling.Studies should also be conducted on the recycling possibility of the sorbent materials.

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CAROB (*Ceratonia siliqua*): HEALTH, MEDICINE AND CHEMISTRY

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Carob (*Ceratonia siliqua*) is one of the important crops over western Asia and North Africa. Its nutritional value has been acknowledged for millennia but its medicinal properties were practically studied only in the last four decades, despite the fact that some of them were used in traditional medicines for centuries. Modern food industry is just starting to discover the great potential of this plant. Carob has outstanding antioxidant capacity along with other important medicinal activities. Some of these have been extensively studied and reported in the last decades, but very few review articles were published about this plant, that summarize and discuss the findings. In this comprehensive review article, we present these reports and discuss them with special attention to traditional medicine, modern research findings, natural products and recommendations for future research subjects.

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Introduction

Carob (*Ceratonia siliqua*) is an evergreen tree that belongs to the legume (Fabaceae) family. Its original habitats are the western parts of Asia, but after its domestication, it spread to all Mediterranean basin and then to the western shores of the Americas, South Africa and southern regions of Australia.[1] Until 1980, the genus *Ceratonia* was considered to include only one species, Carob, *C. siliqua*, but in 1980, another species, *C. oreothauma*, was identified in the eastern region of Africa and Arabian Peninsula.[2] So far, *C. oreothauma* has not been reported to have medicinal or nutritional properties.

According to archeological studies, Carob was used by human beings since very ancient times. But the accurate time of domestication remains debatably unclear. Some scholars reported that it was as late as the Roman era,[3,4] other studies indicate much earlier use.[5,6] However, due to its economic and nutritional value, especially in North Africa, many studies were conducted to investigate the various conditions that effect growth of the plant,[7] and the quantity and the qualities of the fruits (pods) of various wild and domesticated variaties.[8,9]

As far as we could find, three comprehensive review articles were published so far about chemical and medicinal properties of carob. R. Sundararajan and his colleagues meantioned *C. siliqua* among other plants that contain phytoconstituents with nephroprotective activity.[10] The authors designate Carob as a an important plant that has natural products with nephroprotective activity, and they cite "Ben *et al.*, 2011". But reading the reference list revealed no Ben. The article of Nasar-Abbas focuses only on Carob, it is much more comprehensive and more reliable.[11] This article has two weaknesses. First, it does not refer to traditional medicinal knowledge which is the base of many of modern research studies. Secondly, it does not refer to medicinal activities of various extracts and other

Table 1. Ethnomedicinal uses of Carob

Region	Ethnomedicinal use	Method/Reference
Cyprus	Laxative	NI[a],[13]
Egypt	Diarrhea	Infusion[14]
Greco-Arab	Diabetes, herpes, lip sores	Leaf decoction[15]
India	Antiobesity	NI[16]
Iran	Menorrhagia	Patient should sit in a container of a decoction of several plants. Including Carob Details in reference[17]
Iraq	Abdominal pain, diarrhea	Eating ripe fruits[18]
Israel	Antidiabetic; Against viral and bacterial infections and fevers	Leaves decoction: 50 g in 1 L;[19] Fruit syrup mixed with sesame paste[20]
Italy	Animal food; Emollient	Dry fruits,[21] Fruits decoction with *Ficus carica* and Malva sylvestris[22]
Jordan	Antidiabetic; Cough	Leaves decoction,[23] Hot/cold infusion of fruits[24]
Lebanon	Sweetener; medicinal	Molasses of ripe fruits,[25] NI[26]
Morocco	Diarrhea, fish killer, kills intestinal parasites, Relief of skin, digestive system, nervous system	Fruit, bark, leaves,[27] Fruits/leaves decoction,[28] Infusion/decoction of fruit powder, oral.[29] Fruit powder.[30]
Palestine	Food	Fruits eaten raw(cooked) or ripe[31]
Sicily	Food	Fruits eaten raw(cooked) or ripe[32]
Spain	Chocolate, coffee substitute, olive preservative	Ripe fruits, leaves[33]
Tunisia	Food	Ripe pods[34]
Turkey	Diuretic, purgative	Fresh pods[35]

[a]NI= not indicated

materials produced from the tree leaves, which are drawing more and more research attention. Finally, an excellent yet a short review article was published by an internationally leading researcher of Carob, K. Rtibi and his colleagues from Tunisia.[12] But in addition to being a relatively short article, it also focuses on the fruits of the plant, hardly mentioning leaves and other parts of the tree. It also presents all data from the gastrointestinal tract point of view. But it displays some important phenolic compounds in clear and useful figure (page 3 in article).

Ethnomedicinal and other traditional uses of Carob

As mentioned above, Carob is being used by humans as food source and for medicinal purposes since antiquity. Most nations of the Mediterranean basin have such recorded uses as shown in table 1, but other nations discovered its qualities as well.

Modern research of Carob uses, composition and biological activities

Comparing with other fruiting wild trees with very important nutritional value, modern research has neglected Carob. This is to say that most publications of its medicinal/biological activities date from the 2000's. Most of the few earlier publication focus on chemical composition of the pods. We summarized below the findings of modern research of the properties and health uses of Carob. It is important to advise readers that when looking for a specific activity, it is worthy to look for it not only in designated columns, because many publications include reports of more than one activity. Moreover, many publications about Carob are not cited in this review because they studied subjects that are out of the interest of this article.

Antiatherogenic

Aqueous extracts of fruits inhibits lipid peroxidation, inflammation and enhances cholesterol efflux.[36]

Antibacterial, antimicrobial, antifungal and related activities

Methanolic extract of *C. siliqua* was tested for antibacterial activity, compared with methanolic extract of *Plantago major*, which was found more active for most bacteria. The extract of *C. siliqua* was more active for *Enterococcus sp*.[37] Aqueous and methanolic extracts were tested for antibacterial activity, alone and in combination with other antibacterial agents (ampicillin, gentamicin, amikacin and clindamycin). The combination of extracts and antibacterial agents was more efficient than each separately. The extracts were analyzed and some pure compounds were isolated and characterized (Figure 1).[38] Ethanolic and acetone extracts were tested for antibacterial activity against *P. atrosepticum* in potato soft rot. Acetone extract was more active.[39] Methanolic extract of leaves found to be active against *Listeria monocytogenes*. HPLC analysis of extract yielded seven compounds with antibacterial activity, especially epigallocatechin-3-gallate (Figure 2).[40] Dichloromethane-methanol (1:1, v/v) extract of dried pods was tested against 14 types of bacteria and fungi.

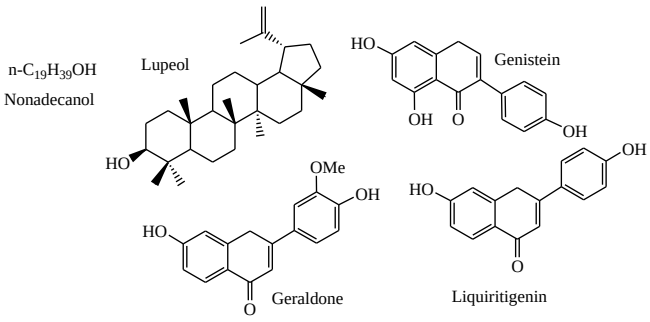


Figure 1. Some compounds isolated from the extract of *C. siliqua*

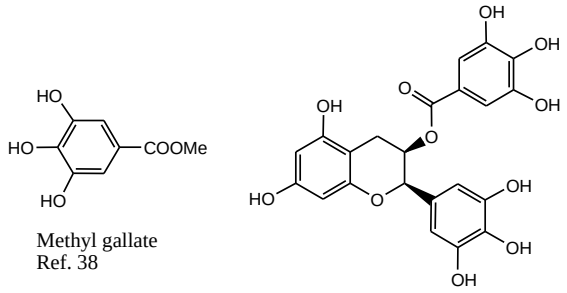


Figure 2. Some compounds isolated from *C. siliqua* by HPLC.

It was found highly active against 11 of them, in concentrations of 1000 and 500 μg/ml.[41] Chloroform and hydroalcoholic (no ratio indicated) extracts of dry leaves were prepared and found active against 15 species of bacteria and fungi, including 3 species of C. albicans.42

Dried powder of the plant (part not indicated) was soaked in methanol and the suspension was centrifuged, filtered and tested for antibacterial and antifungal activities results were positive. Its photosensitizing capacity was also tested and found sufficient. No analysis for natural products was done.[43]

Hexane, chloroform, ethyl acetate and methanolic extracts of dry leaves were prepared and tested for antifungal activity against citrus sour rot agent *G. candidum*. First two were inactive and methanol extract more active than ethyl acetate extract.[44] Methanolic extract of dry pods was prepared and tested against 13 different microbes and 8 different fungi. It was active against all. Total phenolic content was found as 465.5 mg g^{-1} with reference to gallic acid, and total flavonoid content was found as 24.6 mg g^{-1} compared with quercitin.[45] Fresh fruits were extracted with 80 % methanol in water. The extract was found antimicrobial and moderate antioxidant (DPPH).[46] Dry leaves were extracted with n-hexane, ethanol, methanol, ethyl acetate and water. Extracts were tested for antimicrobial, antifungal and cytotoxic activity (brine shrimp assay). n-Hexane was the most cytotoxic and it had the highest antimicrobial activity along with methanolic extract.[47]

Antidiabetic and related activities

Ethanol/water (96 %) extract of dry pods was tested for streptozotocin-induced diabetes in rats. It decreased blood glucose and lipids.[48] Mixture of dried flowers of Roselle (*Hibiscus sabdariffa*) and dry pods of Carob, was water extracted and administered to alloxan-induced diabetic rats. The extract was tested with or without Gamma radiation of the plants mixture powder. In both cases it was found active.[49]

Aqueous extract of immature pod was tested in alloxan-induced diabetic rats for antidiabetic activity. It was found more active than the aqueous extract of mature pods.[50] Powder of dry pods was extracted with n-hexane for phytosterols. The dry extract was found active against alloxan-induced diabetes in pregnant female rabbits.[51] Same extract was used by the same group to test the same activity, but female rabbits were not pregnant.[52] Fiber purified aqueous extract of seed-free dry pods, was prepared and tested for antidiabetic activity by α-glucosidase inhibition. Sufficient activity was found.[53]

Antiviral activity

Ethanolic extract of leaves was tested against Newcastle Disease Virus and found partially active.[54]

Anticancer, antiproliferative and related activities

Ethanolic and ethyl acetate extracts of propolis that was collected in an area (Morocco) with Carob as a major tree were prepared, and tested against three mammalian tumor cell lines. Medium activity was measured.[55] Dry pods were extracted with ethanol, and it was analyzed for reductive components (very detailed), antioxidant, anticancer and anticalpain activities. It was found moderately active for the three activities.[56] Aqueous extracts of dry pods or leaves were tested against mouse hepatocellular carcinoma cell line, and both found active, but leaves extract was more active. Authors attribute this activity to the presence of gallic acid and some of its esters.[57] Methanolic extracts of pods and leaves were prepared and tested for antiproliferative and apoptotic activities in MDA-MB-231 human breast cancer cells, and both found active, with higher activity of leaves extract. Authors relate these activities to the presence of phenolic compounds.[58]

Antidepressant

Acetone extract of fresh pods was prepared and analyzed for tannins content, and tested as antidepressant by tail suspension test and forced swim test. It was active in both and the proposed mechanism of action is by an interaction with the adrenergic and dopaminergic systems.[59]

Antifibrotic

Seed and fibre free dry pods were extracted with water and tested for *Schistosoma mansoni*-induced liver fibrosis and reduction of oxidative stress in mice. Significant activity was found.[60]

Anti-inflammatory and related activities

Methanol extract of bark was tested for antioxidant (DPPH), acute toxicity (rats) and chemical (Carrageenan) or mechanical pow oedema. It was analyzed for major compound families and flavonoids, tannins, sterols, quinones and mucilages were found.[61] A galactomannan extracted from pods, authors refer to as LBG (locust bean gum) was used as constrast material in magnetic resonance enteroclysis (MRE) for immaging of Crohn‘s disease (bowels chronic inflammatory disease). It gave best results when used with water and mannitol.[62] Leaves were extracted with dichloromethane:methanol, 1:1 v/v. The extract was tested for anti-inflammatory and cytotoxic activities and found inactive in both.[63]

Antioxidant and related activities

Methanol extracts of leaves and pulps (all sexes of the tree) were tested for radical scavenging (DPPH) and antioxidant (carotene-linoleate) activities and leaves extract found to be more active.[64] Leaves were extracted successively with hexane, methanol/water (8:2, v/v), diethyl ether, dichloromethane and ethyl acetate. The extracts of three varieties of the tree were tested for antioxidant activity (DPPH) and total phenolic content was determined.[65]

Ethanol extract of dry pods was tested for antioxidant activity (BHA, highly active) and analyzed for polyphenols and carotenoids.[66] 80 % Aqueous methanol extract was prepared from pods and tested for antioxidant activity (3 methods) and analyzed by reverse phase HPLC.[67] Immature pods were extracted (without seeds) with water, methanol, ethanol, acetone, petroleum ether and hexane. Extracts were tested for antioxidant activity (ABTS), analyzed for total phenolic content and tested *in vitro* and *in vivo* (rats) for cerebral and myocardial lipid peroxidation. Polar extracts were more active than non-polar ones.[68]

Methanol extract of leaves was tested for antioxidant activity by various methods and found highly active compared with other fruiting plants.[69] Total phenolic content and antioxidant (DPPH, FRAP) capacity of dry leaves methanolic extract were determined. Compared with other studied plants (3), Carob showed moderate activities.[70] Dry Carob pods powder aqueous extract was prepared and its total phenolic content and antioxidation activity were determined. Both were found relatively high.[71] Aqueous and methanolic extracts were prepared (plant part not indicated) and were tested for antioxidant activity and total phenolic content. Both extracts showed similar results and were relatively high compared with other (94) plants.[72] Methanolic extract of leaves was prepared and tested for radical scavenging activity (DPPH, high), antioxidant activity (linoleic acid system assay, high) and antitumor activity (remarkable). It was also analyzed for polyphenolic content.[73]

Aqueous extract of dry pods was prepared and analyzed for major nutritional materials and minerals. Its antioxidant activity and total phenolic content were determined. An infusion was made that contained Carob extract with other plants and antifungal and antibacterial activities were measured and found high.[74]

Methanolic and ethyl acetate extracts were prepared from bark and were analyzed for major compound families, total phenolic content and antioxidant activity (DPPH) were measured. Methanolic extract was more active.[75] Dry leaves were extracted with 80 % ethanol-water, and the extract was fractionated with n-hexane, DCM and ethyl acetate. The ethyl acetate fraction showed the highest antioxidant (DPPH, β-carotene bleaching assay) and antimicrobial activities.[76] Carob seeds were ground and supplied, without further treatment, to female rats to test antioxidant and hepatoprotective activities of the powder against ethanol-induced oxidative stress. Results were measured by serum enzymes.[77] Aqueous extract of dry pods (or leaves) was prepared and found active inhibitor of neutrophils myeloperoxidase.[78,79] Dry leaves aqueous extract was found protective against cell DNA oxidative stress caused by H_2O_2 and it had no genotoxicity.[80] Fresh pulp was extracted by ultrasonic-assisted 80 % aqueous ethanol. The effect of the free/ecapsulated extract was tested for anioxidant and nutritional value of yogurts. The free form had higher antioxidant activity (shorter shelf-life) and both forms did not alter nutritional value.[81] Ground pods were defatted with hexane and the dry residue was added to Kefir (yogurt). It improved its antioxidant capacity and increased bacteria growth.[82] Dry pods powder was added to pasta flour. The antioxidant activity (ABTS, FRAP) was improved and the nutritional values were increased.[83] Powdered Carob pods were added directly to rats food to test its hepato- and nephroprotective effect against oxidative stress induced by CCl_4. Positive results were obtained.[84] Pulp powder was extracted with acetone/water (7:3) and encapsulated with polycaprolactone. Encapsulated and non-ecapsulated extract of ripe and unripe pulp was tested for antioxidant activity (ORAC, DPPH, FRAP) after *in vitro* digestion model. The encapsulation provided slow release of phenolic compounds and protection against digestive fluids.[85] Pod powder was fed to rabbits and its antioxidant activity was monitored through the activity of three oxidizing enzymes. It also promoted the growth of the animals.[86]

Antiatherosclerotic

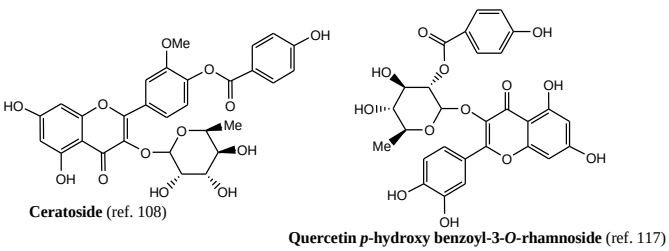
Commercial insoluble dietary fibre (81 % w) from carob pod was found antiatherosclerotic in rabbits.[87]

Anxiolytic-sedative and antiproliferative

Methanolic extracts of leaves and pods were prepared and tested for central and peripheral benzodiazepine receptors binding. Leaf extracts, especially young leaves, found more active. No active compounds are indicated.[88]

Chemical composition and some related activities

Methanolic and aqueous extracts of sapwood were prepared and analyzed for phenolic contents and their components. The extracts were also tested for antioxidant (H_2O_2) and antitumor activities, where methanolic extract was more active.[89] Pods of trees from various locations in Morocco were analyzed for fibre, sugar and polyphenolic content.[90] Seeds-free pod syrup was prepared from trees that grow in Bulgaria and in Turkey, analyzed for sugar content and compared. Turkish product had higher sugar content.[91] Chemical composition of pods from different localities was determined for major compound families, minerals and nutritional ingredients. Aqueous extracts were prepared with hot and cold water, and higher temperatures yielded higher concentrantions.[92] Determination of the tannins, pectins, hemicellulose, cellulose, nitrogen, mineral elements, sugars and fat contents was carried out on carob pods.[93] Major chemical composition was determined with detalied extraction (aqueous) conditions of time and temperature.[94] Composition of Carob pods was determined in terms of carbohydrates, protein, fat, polyphenols and tannins. 70 % acetone/water was the most effective solvent for the extraction and recovery of tannins.[95] Detailed report of major nutrients determination in pods, with results and methods.[96] Pods were analyzed for carbohydrates, protein, fat, minerals, vitamins (6), phenolics (11) and fatty acids (17).[97] Major nutritional components of pulp and detailed amino acid composition is reported.[98] Methanolic extract of green pods and leaves was prepared, analyzed for chemical composition (major categories) and tested for antibacterial activity.[99] Three pulp extracts were prepared: 70% ethanol/water, 80 % methanol and aqueous. All were analyzed for chemical composition and tested for *in vitro* antioxidant activity. Ethanolic extract found most active and it was tested *in vivo* (rats, $AlCl_3$-induced oxidative stress), and found active antioxidant.[100] This study focused on carbohydrate and fatty acid content of pods during different development satges of Carob trees. It also compared wild and domestic cultivars.[101] This study compares between phenolic content (mainly) of ripe and unripe pods.[102] Effect of seasonality and its relation with CO_2 assimilation and photosynthesis is reported in terms of changes in chemical composition of major nurtrients.[103] Detailed study of determination of compounds in methanolic pod extract by Liquid Chromatography–Electrospray Ionization-Tandem Mass Spectrometry. Details include conditions (solvents, positive, negative), compounds and their fragmentation.[104] Essential oil was prepared from fresh whole pods and extracted from aqueous phase with DCM. Its analysis showed mainly long chain hydrocarbons, unpolar acids and other unpolar compounds. It was found active against several types of bacteria and cycotoxic against cancer cell line.[105] A short review article about chemical composition of Carob with some interesting uses that are usually ignored by other reports has appeared.[106] Total flavonoid content of leaves was determined by 70 % ethanol/water extraction. The extract was analyzed by HPLC and nine compounds were detected, with myricetin as major.[107] New acylated flavonol glycoside was isolated and characterized from methanolic extract that was fractionated and chromatographed by various solvents. Authors name the new compound ceratoside (Figure 3). Full spectroscopic data is reported.[108]



Ceratoside (ref. 108)

Quercetin *p*-hydroxy benzoyl-3-*O*-rhamnoside (ref. 117)

Figure 3. New compounds isolated from Carob.

Experimental and theoretical method for reducing isobutyric acid content in pods by roasting is required, since its foul smell decreases Carob uses for food products.[109] Seed oil was analyzed and the major constituents are: fatty acids, tocopherols and phytosterols.[110] Defatted pods of two types (natural and commercial flour) were extracted with methanol and phenolic acids content was determined. Gallic acid was the major constituent.[111]

Dry leaves were extracted with 70 % ethanol/water and analyzed by different methods for polyphenols. No new compounds are reported.[112] Leaves, bark, skin and pulp were oven-dried (500 °C) and their mineral content (Ca, K, Mg, Na, P, Cl, Cu, Fe, Zn, Se) was measured by atomic absorption spectrophotometry.[113] Nitrogen and ash content of pods was measured in wild and grafted Carob trees.[114] Mineral content (Mg, Cu, Fe, Zn, Se, Mn) of pods was determined by ICP-OES.[115] Partial mineral content (K, Ca, Cl, Mg, Na) was determined by neutron activation analysis.[116] Kinetic study of the extraction of polyphenols from pods using environment friendly solvents (best, 30 % ethanol/water, 1 % citric acid), and a new compound reported (Figure 3).[117]

Three types of domesticated Carob were analyzed for sugars, minerals and fatty acids.[118] Phytochemical analysis of methanolic extract of pods has been done and antioxidant (DPPH) and cytotoxic activities were tested. Polyphenolics (gallic acid) comprised most of the extract, that had high antioxidant activity and low cytotoxic activity.[119] D-pinitol content in various commercial Carob syrups was determined to be around 90 g Kg^{-1} on an average of the dry weight.[120] A detailed report of isolation, quantification and identification of polyphenols in pods has appeared.[121] 70 % Acetone/water was used to extract pods and the extract was analyzed for polyphenols and tested for antioxidant activity. Gallic acid was major compound.[122] A short report of quantification of polyphenolic content of different Carob cultivars in Portugal has been published.[123] Total phenolic content of pods from different areas and different domesticated cultivars in Morocco was determined in this report.[124]

Comparison between ripe and unripe pods and using three solvents for extraction of the content of leucoanthocyanins (3,4-dihydroxy flavans).[125] In this report, 137 compounds were identified in the volatile phase of Carob seeds. During roasting, more than 50 % of them are evaporated, mostly those that have unpleasant odors.[126] Later study discovered 169 compounds with clear dominance of short chain acids (77.5 %).[127] Use of advanced isolation and detection method (headspace solid-phase micro extraction, HD-SPME) revealed new yet known volatile compounds in pods, that were not reported in ref. 126, 127.[128] The extraction, isolation, quantification and characterization of galloyl glucose compounds have been reported.[129]

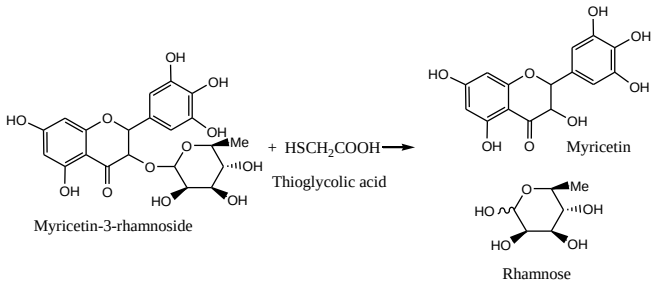
Deseeded pods of cultivatied and wild varieties were analyzed for sugar content. Domesticated plants had higher sugar content. Monosaccharide compositions were also reported.[130] Alkaline hydrolysis of condensed tannins separated sugars from polyphenols in pods, gave (+)-gallocatechin, (-)-epicatechin and (-)-epicatechin gallate as major compounds.[131] Tannins isolated from ripe pods were hydrolysed with thioglycolic acid to yield free polyphenols and sugars (Scheme 1).[132] It has been claimed that part of sweet taste of pods is due to 6-deoxymannose and 1-methoxy-6-deoxygalactose but no evidence has been provided.[133]

Ethanol production

Ethanol was produced from pods that were extracted with water. Details of extraction and fermentation conditions have been presented. Maximum yields were around 45 %.[134]

Heavy metals related activities

Silver nanoparticles were prepared from $AgNO_3$ solution, using Carob aqueous extract as reductant. The AgNP's have antibaterial activity against *E. coli*.[135] Dry pod methanolic extract found to be active corrosion inhibitor of copper and brass in aqueous 1 M HNO_3.[136] Bark was cleaned and treated for biosorption of heavy metals (Zn^{+2}, Ni^{+2}, Cu^{+2} and Cd^{+2}). Different variables are discussed and pH, metal concentration, contact time, adsorbent dose have been optimized. Kinetics of adsorption has been presented.[137] Ethanol extract of pods was found protective against lead (Pb) poisoning in *Oreochromis niloticus* fish. In this report, all along the article ether extract is mentioned but in the experimental section, alcohol extraction is described.[138]



Scheme 1. Hydrolysis of tannins.

Human nutrition, nurtitional sources and risk assessment

Carob flour was added to Tarhana (yogurt-wheat based food), and fibre, ash, Ca, K, Cu, total phenolic content and total antioxidant capacity were determined. All nutitional values and antioxidant property increased.[139] Powder of Carob pods was added to wheat biscuits. Their mineral and total phenolic content was found higher than biscuits without addition.[140] Risk assessment (Italy) of toxic metals (Pb, Cd) and pesticides (organochlorines, organophosphates, pyrethrins and pyrethroids, in honey of bees that feed on Carob trees) were done. No risk was detected.[141] Heavy metals (Cd, Pb, Fe, Cu, Mn, Ni, Cr, Se) in honey of bees that fed from Carob trees (Turkey) among other plants have been determined.[142]

Honey of bees that feed on Carob flowers (Italy) has been physicochemically characterized. Most important presented value is sugar content.[143] Honey of bees that feed on Carob flowers (Morocco) was physicochemically characterized. Most important presented value is mineral content. Antioxidant activity (DPPH) was tested and found high.[144] Human volunteers, fed with tannates rich carob-fibre that was added to diary food, had improved cardiovasular functions and significantly lowered cholesterol levels.[145] Extraction of pods with refluxing methanol resulted in high

content of condensed tannins extract, that was added to kids' diet. Lowering of cholesterol level was significant.[146] Sancks that conatined Carob with the same amount of available carbohydrates as chocolate snacks were compared in terms of glycemic index (GI). Carob snacks resulted in much lower GI.[147] This study aimed on the finding of optimal ratio of Carob flour in snack to achieve acceptable taste and texture in order to compete with chocolate snacks.[148] Nutritional content was tested and compared between homemade and commercial Carob flour. In all nutritional aspects, soluble sugars, amino acids, fatty acids and mineral content, homemade flour was better.[149] Carob pods are fermented in to produce a special type of wine in Serbia.[150]

Animal food and nutrition

Aqueous extract of Pod was tested against cellulolysis and proteolysis of rumen bacteria activity. Its inhibition of cellulolytis was related by authors to high carbohydrate content, while anti-proteolysis activity was related to tannin compounds.[151] Deseeded pods and seeds were analyzed for mineral, protein and energy content. Seeds have higher values but tests showed that they are harder to digest for animals. So, pods have higher nutritional importance.[152] Sugar content of Carob pods help both nursing ewes and lambs in the growth process.[153] Effect of feeding quails with Carob seed powder as food additive was tested. It was found that mortality of birds decreased, egg quality and fatty acid content increased, and cholesterol levels decreased.[154]

Gastroprotection and other digestive system related activities

Pods were extracted with 70 % methanol/water and extract was tested against HCl-ethanol induced gastric ulcer and found moderately active, compared with other plants used in this study.[155] Aqueous extract of pods was tested for antioxidant activity (DPPH) and against ethanol-induced oxidative gastric stress (lipoperoxidation and hydrogen peroxide increase) in rats and found active.[156] Aqueous extract of pods was tested against dextran sulfate sodium-induced sub-acute experimental ulcerative colitis in adult Wistar rats. Results were positive and authors attributes this to phenolic compounds in Carob.[157] Aqueous extract of pods was prepared, analyzed (RP-HPLC) for tannins and dietary fiber, total sugar and total phenolics content. It was also tested against small intestinal motility in rats and jejunal permeability in mice, and found active in both tests.[158] Soluble galactomannans were water extracted from Carob seeds, and formulated to treat gastric reflux of infants. Results were positive after three days treatment.[159]

Hepatoprotective

Aqueous extract of dry pods was tested against ethanol-induced oxidative stress in rat liver and found active.[160]

Inhibition of enzymes and other medicinally active materials

Ethyl acetate extract of immature pods inhibits the growth of peas (*Pisum sativum*) promoted by gibberellic acid.[161] Growth inhibition of peas by different extracts of Carob was compared with the same activity of abscisic acid. This natural growth inhibitor is stronger than Carob extracts.[162] Carob extracts inhibit natural growth promoter indol-3-acetic acid, but this inhibition is weaker than inhibition of gibberellic acid (ref. 161).[163] Pods were extracted with 60 % ethanol/water and the extract moderately inhibited protease action. This was done in search of anti-infectous plant extracts.[164] Decoctions of different parts of Carob tree were prepared and tested for antioxidant activity (DPPH, ABTS and FRAP), and enzyme inhibition (AChE, BuChE, α-amylase and α-glucosidase). All decoctions were highly active in all tests.[165]

Nephroprotection and urinary system related activities

Carob honey (syrup) was tested as diuretic in rats and found very active. Authors attribute this activity to flavonoid content of Carob pods.[166] Pods were extracted with 70 % ethanol/water and the extract was administered to diabetic (STZ-induced) rats. Results showed moderate improvement in kidney function.[167] Aqueous extract of pods was prepared and tested against dextran sulfate sodium-induced injuries in rat liver and kidney, and found active. Polyphenolic content of extract is probably responsible for the antioxidant and anti-inflammatory properties.[168] Leaves and pods were extracted with 70 % ethanol-water, and the extract was tested for reduction of oxidative nephrotoxicity of synthetic anticancer agent cisplatin, and found active.[169] Leaves were extracted with 80 % ethanol/water and the extract was fractionated with ethyl acetate, n-hexane and DCM. All fractions, including the remaining aqueous phase, were analyzed for total phenolic content and tested for antioxidant activity (3 methods). It was also against renal failure caused by oxidative stress that was CCl_4-induced. All tested fractions were active, where the ethyl acetate fraction was most active.[170]

Glycemic response

Carob seeds were extracted with methanol and petroleum ether. Both extracts were tested *in vivo* (rats) for glycemic response. Crude polyphenols of Carob were also tested. No positive results were obtained.[171] Healthy human volunteers were supplied with tablets of Carob flour. Glycemic index showed clear improvement.[172]

Neuroprotection

In a short review article attempts have made to link the active compounds in Carob with neuroprotection against monosodium glutamate damages. No direct link is established.[173]

Reproductive system related activities

Monosodium glutamate caused damage to reproductive organs of female Wistar rats. When fed with Carob powder, the damages were less.[174] Pods were extracted with 50 % ethanol/water and the extract was tested for its effect on fertility of male rats. Results indicate that it can increase testosterone synthesis and increases sperm density in seminiferous tubules.[175] A review article mentions Carob as a medicinal plant (decoction) for treatment of abnormal uterine bleeding by Avicenna.[176]

Phytoestrogens, bone recovery

Seed flour was screened for chemical composition, especially lignans. The detected phytosterols were secoisolariciresinol, lariciresinol, isolariciresinol and pinoresinol (Figure 4).[177] Rats that were fed with Carob pod powder showed improvement in osteoporosis bone model in rats.[178]

Skin depigmentation

Various parts of Carob were extracted with ethanol and the dry extract was fractionated with other solvents. Methanolic fraction of bark extract was most active inhibitor of tyrosinase, the enzyme responsible for oxidation of amino acid tyrosine, that causes hyperpigmentation. The extract was also tested in L-dopa model and found active. It was also tested with human volunteers for irritation and found non-irritant. Three isolated gallates were active.[179]

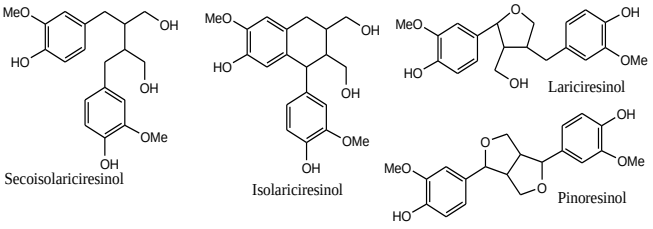


Figure 4. Phytosterols detected in the seed flour of Carob.

Toxicological evaluation

Goats fed with Carob leaves showed no signs of illness.[180] Seeds aqueous extract was prepared and supplied to rabbits in drinking water. No physical or behavioral adverse effects were recorded.[181]

Meatbolism of proanthocyanins

Proanthocyanidins (condensed tannins) in unripe pods were radiolabelled by ^{14}C, they were extracted with acetone-water (3:1 v/v), isolated and fed to rats. Proanthocyanidins were partially metabolized and they are not inert within the animal gut.[182]

Neutralization of toxicity of Egyptian horned viper venom

Aqueous extract of seeds was tested for neutralization of toxicity of Egyptian horned viper (*Cerastes cerastes*) venom *in vitro* (hyaluronidase inhibition) and *in vivo* (rats). It had weak activity.[183]

Waterpipe smoke and amiodarone toxicity amelioration

Aqueous extract of pods ameliorates impairments in liver, kidney and lung functions and decreased the oxidative stress induced by exposure to water pipe smoke and amiodarone in rats.[184]

Discussion

Reading the literature of ethnomedicinal and other traditional uses of Carob (Table 1) reveals an interesting, yet a strange fact. The plant (molasses of ripe pods) is very well know in Palestinian traditional medicine to have strong anti-inflammatory activity, especially mouth inflammations; and modern research approves this property. But articles of ethnomedicine of Carob do not mention this at all. S. A. Baydoun and her colleagues from Lebanon (Ref. 26), mention many traditional uses of Carob but not a clue of anti-inflammatory activity.

Antibacterial/antifungal activity of various extracts of *C. siliqua* was reported by many research groups (ref. 38-47). A. H. Ibrahim and her colleagues, prepared aqueous and methanolic extracts of *C. siliqua* and tested them for antibacterial activity.[38] They isolated nine pure compounds from these extracts. All the nine compounds are known to have antibacterial activities. The structures of five of them are shown in Figure 1.

These five compounds were reported by other groups for having antibacterial activities. For example, n-nonadecanol from *Schinus lentiscifolius*,[185] lupeol from *Albizia adianthifolia*,[186] genisstein from soybean (*Glycine max*),[187] geraldone from *Flourensia oolepis*,[188] and liquiritigenin from *Dalbergia odorifera*.[189] N. Aissani *et al*. isolated (−)-epigallocatechin-3-gallate (Figure 2) from the methnolic extract of Carob leaves. It is interesting to notice that in reference 38 the same extract was prepared and analyzed but this compound was not isolated. Methyl ester of gallic acid was isolated. Epigallocatechin-3-gallate is the major antibacterial agent in green tea (*Camellia sinensis*).[190]

One general fact can be noticed, methanolic extract of leaves has the highest antibacterial capacity. But it is also important to notice the contradiction between the reports of I. Talibi *et al*.[44] and B. Kivcak and T. Mert[47]: while the first reports that n-hexane extract of dry leaves was inactive antimicrobial, the second reports that the very same extract had the highest activity, along with methanolic extract. The weak antimicrobial activity reported by C. Tassou *et al*.,[191] can not be explained or compared with later reports since Carob tree part was not indicated and extraction solvent was 80 % ethanol/water.

But Carob can be a medium for bacteria growth also. A. Hariri and his colleagues reported the growth of *L. bulgaricus* from Carob pods syrup.[192] Despite the fact that this bacterium is a "friendly" one that is used for cheese production from milk, the sugar rich Carob pods syrup, that has high nutritional value, can potentially help growing harmful bacteria. Fungi can also grow on nutritional Carob trees. M. El-Neketi and her colleagues have isolated and characterized six new interesting compounds, including alkaloids and polyketides from the fungi *Penicillium citrinum*, that grows on Carob trees in Morocco.[193] The structures of these compounds are shown in Figure 5.

Among the reported Carob extracts tested for antidiabetic activity, it seems that the report of F. Mounce and M. Al-Saeed (ref. 51) is the less understandable.

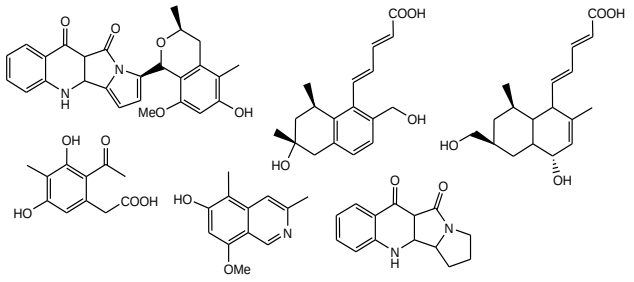


Figure 5. Alkaloids and polyketides from the fungi *Penicillium citrinum*, that grows on Carob trees.

They claim to find n-hexane extract of dry pods to be active against alloxan-induced diabetes in pregnant female rabbits. They claim that this extraction method yields "phytosterol extract". Obviously, phytosterols are highly soluble in hexane, but this extract naturally contains many other non-polar compounds that are found in Carob pods. So, it is scientifically inaccurate to refer the biological activity to a single compound or compound family, while the extract is crude and not fractionated.

Anticancer and related activities of Carob have been studied partially. Anyhow, some of these published studies must be considered very carefully. For example, H. A. Mouse and his colleagues tested ethanolic and ethyl acetate extracts of popolis extract from Morocco. They claim that the raw material was prepared by bees from mailny three trees, including Carob. But when they analyzed these extracts by HPLC, they did not find a single compound that is solely conatined in Carob.

Anti-inflammatory activity of Carob extracts reported in references 61-63, are to the best our knowledge, underestimating the capacities of this plant. Based on very pesonal experience and many others, we can state that molasses of Carob are very active against *mouth* infalammations. As we mentioned above, this is very well known activity in Palestinian traditional medicine, and its very strange that even publications about ethnomedicine of *C. siliqua* do not mention it.

Most reports about medicinal activities of Carob extracts, especially antioxidant activity and its related properties (ref. 64-86) indicate very clearly that extracts of polar solvents have higher activity than those of non-polar solvents. In most cases, extraction was done with methanol. This can be understood on the basis of the nature of the active compounds in Carob, most of them are polyphenols, that are relatively polar compounds. Even though other powerful antioxidants such as carotenoids are present in Carob, their amounts are much lower than that of polyphenols, and so, their contribution to the total antioxidant capacity is low. Special attention can be paid to the report of A. Ben Hsouna and his colleagues (ref. 76), where they state that the ethyl acetate leaves "extract" had the highest antioxidant and antimicrobial activities among the "extracts" that they prepared. As far as we can understand this, introducing the results this way is misleading. Leaves were originally extracted with 80 % aqueous ethanol, a very polar solvent. The dry extract was fractionated with ethyl acetate, DCM and n-hexane, where ethyl acetate is the most polar solvent among the three. HPLC analysis of this fraction yielded polyphenolic compounds: 1,6-di-galloyl-glucose, 1,2,6-tri-galloyl-glucose, myricetin glucoside, 1,2,3,6-tetra-galloyl-glucose, myricetin rhamnoside and syringic acid. To conclude this part, it is important to pay attention to two additional reports. First, S. Klenow and her colleagues,[194] raised the question: does an extract of Carob have chemopreventive potential related to oxidative stress? And after a fundamental research they conclude that the answer is not clearly "yes". So, the antioxidant quality of Carob is superb but its anticancer properties are not the same. This doubt is strengthened by the report of N. Khalifa and her colleagues who found high antioxidant of extracts of leaves and pods of Carob, but zero and very weak cytotoxic activity, respectively.[195*] Second, V. Goulas *et al*. published a comprehensive review article about "functional components of Carob fruit".[196] Its not clear why the most important medicinal and food value of Carob pods, antioxidant activity, was so clearly ignored there.

The chemical composition of Carob was widely investigated. Locality, seasonality and stage of development can influence the chemical composition of plants, and Carob is not an exception (ref. 90-92, 101-103). These effects are well known for other plants, and many studies of Carob indicate this, including the report of A. Haddarah and her colleagues from Lebanon.[197] One of the important reports of chemical composition of Carob was published by Y. M. Boufdi and her colleagues.[100] They prepared three extracts of pulp, and while the vast majority of composition studies report approximate compositions, this study reports a very detailed composition (Table 2, page 79 in the article). Most of the identified compounds are polyphenols and their derivatives.

Polyphenolic compounds are major components of Carob. They are responsible for most of its medicinal and other activities, such as antioxidant-reductive activity. This property enable extracts of Carob being corrosion inhibitors.[136] One of the new polyphenolic that were isolated for the first time from Carob is ceratoside, that its structure is shown in Figure 3.[108]

Hydrolysis (degradation) of condensed tannins which are polyphenols attached by glycosyl bonds to sugars can be done in several reactions. One of the convenient reactions that is performed under mild conditions, is hydrolysis with thioglycolic acid, that can also hydrolyse other ether bonds.[132] This reaction was discussed by Sears and Casebier.[198] In scheme 1, we illustrate the application of this reaction to myricetin-3-rhamnoside.[102]

Carob extracts are being studied in recent years for gastroprotective activities, and they were found highly active, so far.[155-159] Most studies were done *in vivo* after induction of gastric disorder in animals. These diorders were induced by various chemicals such as ethanol, hydrochloric acid, sodium chloride or dextran sulfate sodium. In most reports, authors link the medicinal activity to polyphenols found in Carob pods. One of these compounds is pyrogallol (1,2,3-trihydroxy benzene).[158] A short summary of these activities can be found in a recent review article that was published by I. C. Theophilou and her colleagues.[199] Some activities however, have been attributed to soluble galactomannans.[159] A general structure of Carob galactomannan, polysaccharide composed of mannose in major chain, with galactose branching, is shown in figure 6 (short form).[200]

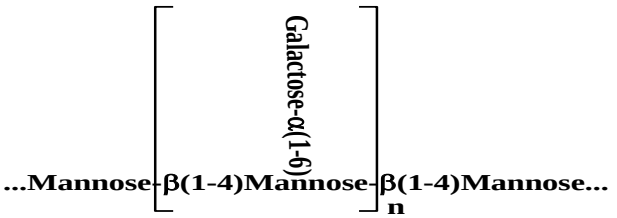


Figure 6. General structure of galactomannan found mainly in Carob beans

Carob phytoestrogens were found active in bone recovery.[177,178] These compounds are shown in figure 4. This activity is very well known and it was finely presented in a comprehensive review article by Lagari & Levis.[201]

Finally, Carob tree is a very important nutritional source, both for humans and animals, and relying on its potenial and safety, even its used parts and by-products can have nutritional and medicinal important uses.[202] It is important to indicate here that in this review article we tried to focus our attention on major natural products found in Carob, despite the fact that some compounds that are found in trace amounts, might be of some interest.[203] But these compounds are found in other plants in higher amounts and were studied in that context.[204]

Conclusions and suggested future research

(1) Carob has very important nutritional and medicinal qualities. Some were reasonably studied but others should be studied more thoroughly.

(2) Some of the reports concerning antibacterial activity of Carob are contradicting. Further studies are needed to clear these confusions. It is also recommended to use modern tools such as ionic liquids for extraction.

(3) There is a need to perform more studies of antiviral capacity of Carob.

(4) It is highly desirableto expand the research of anti-inflammatory activity of Carob since it is very well based in Palestinian ethnomedicine and almost was not studied so far.

(5) Works of actual research of neuroprotective effect of Carob were never published. This field should be extensively studied.

6) There is an importance to expand the studies of the medicinal studies of the potential of Carob products as nutritional sources, especially in terms of antiobesity agents.

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1-(N-ETHOXYCARBONYL-N-ISOPROPYLOXY)AMINO-4-DIMETHYLAMINOPYRIDINIUM CHLORIDE. SYNTHESIS AND STRUCTURE

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Keywords: N-alkoxy-N-chlorocarbamates, 1-(N-alkoxy-N-alkoxycarbonyl)aminopyridinium salts, 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-dimethylaminopyridinium chloride, synthesis, structure.

Stable 1-(N-alkoxy-N-alkoxycarbonyl)amino-4-dimethylaminopyridium salt, 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-dimethyl-aminopyridinium chloride, has been synthesized for the first time. Its structure has been studied by XRD method.

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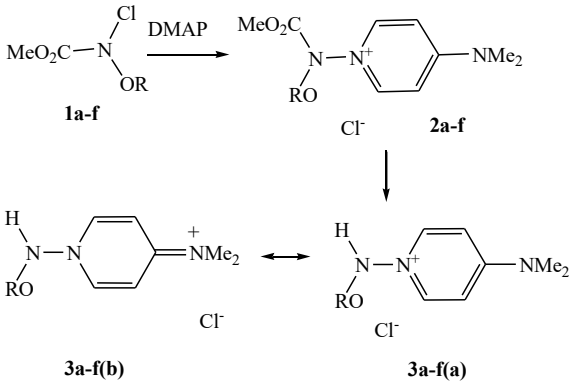
INTRODUCTION

It has been found earlier[1,2] that an interaction of methyl N-alkoxy-N-chlorocarbamates (**1a-f**) with 4-dimethyl-aminopyridine (DMAP) is a route to unknown 1-N-alkoxyamino-4-dimethylaminopyridium chlorides (**3a-f**) which was presumably realized via initial formation of unstable 1-(N-alkoxy-N-methoxycarbonyl)amino-4-dimeth-ylaminopyridium chlorides (**2a-f**) as the reaction intermediates (Scheme 1). Evidently the decomposition of unstable compounds **2a-f** yields 1-N-alkoxyamino-4-dimethylaminopyridium chlorides (**3a-f**). Compounds **3a, b** exist as structure (**3a**,**b (b)**) and "quinonoid" deformation of the pyridine ring take place.[1]

In this article synthesis of stable 1-(N-alkoxy-N-alkoxycarbonyl)amino-4-dimethylaminopyridium chloride and XRD study of its structure have been described.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on VARIAN JEMINI 400 spectrometer (400 and 100 MHz, respectively) in DMSO-d_6 and $CDCl_3$ as solvents with TMS as internal standard. Mass spectrum was recorded on VG 70-70EQ mass spectrometer in fast atom bombardment (FAB) mode. XRD structural study was performed on Xcalibur 3 automatic four-circle diffractometer (MoKα-radiation, graphite monochromator, Sapphire-3 CCD-detector, ω-scanning). DMAP was sublimated under vacuum (3 mm Hg). The solvents were purified and dried according to standard procedures.



R = Me(a), Et(b), i-Pr(c),n-Bu(d), Oct(e), Bn(f)

Scheme 1. Reported synthesis of 1-N-alkoxyamino-4-dimethylaminopyridium chlorides.

Synthesis of 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-dimethylaminopyridinium chloride (5)

A solution of DMAP (166 mg, 1.357 mmol) in MeCN (13 mL) was added to a solution of ethyl N-chloro-N-isopropyloxycarbamate (**4**)[3] (245 mg, 1.347 mmol) in MeCN (6 mL) at 8 ºC. The reaction solution was maintained at 8º C for 46 h, then it was evaporated under vaccum (20 mm Hg), dried under vacuum (4 mm Hg), washed with benzene (12 mL), dried under vacuum (4 mm Hg), giving **5** as colorless hygroscopic crystals (399 mg, 97 %). m.p. 103-104 °C (with decomp.), after crystallization (CH_2Cl_2-EtOAc) m.p. 104-105 °C (with decomp.). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.18 (6H, d, ^3J = 6.0, NOCH<u>Me</u>$_2$), 1.26 (3H, t, ^3J = 7.2, CO_2CH_2<u>Me</u>), 3.36 (6H, s, NMe_2), 4.23 (H, sept, ^3J = 6.0, NO<u>CH</u>Me$_2$), 4.28 (2H, q, ^3J = 7.2, CO_2<u>CH_2</u>Me), 7.39 (2H, d, ^3J = 8.0, H Py), 8.17 (2H, d, ^3J =

8.0, H Py). ^{1}H (400 MHz, $(CD_3)_2SO$) δ = 1.23 (6H, d, ^3J = 6.4, NOCH<u>Me</u>$_2$), 1.26 (3H, t, ^3J = 7.0, CO_2CH_2<u>Me</u>), 3.29 (6H, s, NMe_2); 4.29 (H, sept, ^3J = 6.4, NO<u>C</u>HMe$_2$), 4.30 (2H, q, ^3J = 7.0, CO_2<u>C</u>H_2Me), 7.11 (2H, d, ^3J = 8.0, H Py), 8.68 (2H, d, ^3J = 8.0, H Py). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.9 (CO_2CH_2<u>Me</u>), 20.5 (NOCH<u>Me</u>$_2$), 41.2(NMe_2), 65.7(NO<u>C</u>HMe$_2$), 79.2 (CO_2<u>C</u>H_2Me), 155.6, 156.8 (C-3, C-5, C-2, C-6 Py), 166.7 (C-4 Py), 185.5 (C=O). Mass spectrum, *m/z*, (I_{rel} %): 268 M^+ (100). Anal. Calcd. for $C_{13}H_{22}ClN_3O_3$: N 13.83; Found: N 13.65.

XRD structural study of compound (5)

Crystals of **5** suitable for X-ray structural analysis were grown from a solution in CH_2Cl_2-EtOAc mixture at 6 ºC. Triclinic, $C_{13}H_{22}N_3O_3^+Cl^-•H_2O$, at 100 K, *a*= 9.2359(8) Å, *b* = 13.2566(8) Å, *c* = 13.8685(9)Å, *α* = 75.552(5)°, *β* = 89.534(7)°, *γ* = 89.291(6)°,*V* = 1644.2(2) Å^3, M_r = 321.80, *Z* = 4, space group $P\bar{1}$, d_{calc}= 1.30 g cm^{-3}, μ(MoKα) = 0.25 mm^{-1}, *F*(000) = 688. Cell parameters and intensities of 11451 reflections (5737 independent reflections, R_{int}= 0.068) were measured using «Xcalibur 3» diffractometer (graphite-monochromated MoKα radiatione, CCD detector, *ω*-scan, 2 θ_{max}= 50°).

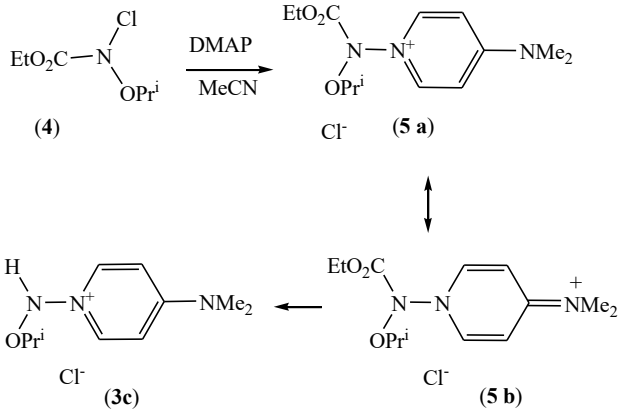
The structure was solved by direct method using SHELX-2016 program package.[4] Positions of hydrogen atoms were located geometrically and refined using the riding model with U_{iso}=nU_{eqv} of the carrier atom (*n*=1.5 for methyl moieties and *n*=1.2 for other hydrogen atoms). Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms to wR_2 = 0.2844 for 5737 reflections (R_1 = 0.108 for 4016 reflections with F>4σ (F), S = 1.389). The final atomic coordinates, molecular geometry parameters, and crystallographic data of compound **5** were deposited in the Cambridge Crystallographic Data Center, 12 Union Road, CB2, 1EZ, UK (fax:+44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk) and is available on request quoting the deposition number CCDC 1565981).

RESULTS AND DISCUSSION

We have found that ethyl N-chloro-N-isopropyloxycarbamate (**4**)[3] reacted with DMAP in acetonitril yielding relatively stable 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-dimethylaminopyridinium chloride (**5**) (Scheme 2). The reaction must be carried out in mild condition at 8 ºC, because under certain conditions compound (**5**) may be spontaneously converted to 1-N-isopropyloxyamino-4-dimethylaminopyridium chloride (**3c**), for example if the reaction time is increased to 93 h.

Compound (**5**) is the first example of stable 1-(N-alkoxy-N-alkoxycarbonyl)amino-4-dimethylaminopyridium chlorides, a novel kind of N-alkoxyhydrazines.[1,2,5-10] Earlier it was regarded that 1-(N-alkoxy-N-alkoxycarbonyl)amino-4-dimethylaminopyridium chlorides were very labile and cannot exist, for example compounds **2a**-**2f**. The structure of 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-dimethyl-aminopyridinium chloride (**5**) has been confirmed by data of ^{1}H and ^{13}C NMR spectra and mass spectrum. Also XRD study of N-alkoxyhydrazine (**5**) has been done (Figure 1, Tables 1 and 2).

It was found that in independent part of the unit cell of crystal of compound **5** there were two organic cations of forms **5A** and **5B**, two Cl^- anions and two molecules of water. Cations **5A** and **5B** differ by some geometric parameters.



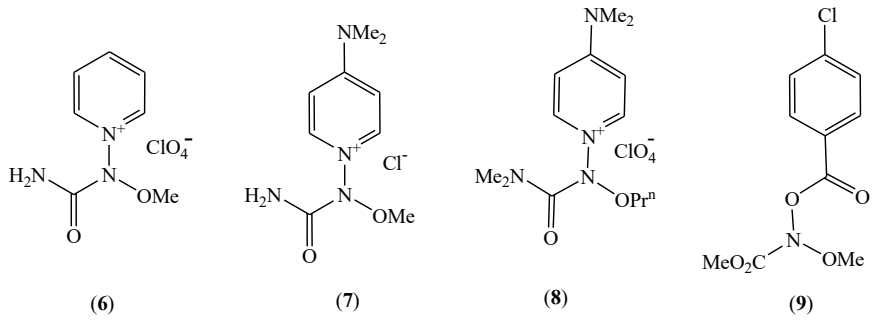
Scheme 2. Formation of (**5**).

Evidently, the positive charge is localized mainly on N(3) atom of Me_2N-group conjugate to the pyridine ring, which is confirmed by shortening of the N(3)–C(3) bond as in cation **5A** and **5B** (Table 1) in comparison with average value of 1.371 Å for N–C_{arom} bond.[11] The lengths of the N(3)–C(3) bonds are close to mean value of N=C bond (1.316 Å).[11] Thus 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-di-3methylaminopyridinium chloride (**5**) structure is more closed to "quinonoid" structure **5b** (Scheme 2).

The observed altering of pyridine bonds conforms to this assumption: the C(2)–C(3) and C(3)–C(4) bonds are elongated in cation **5A** and **5B**, the N(2)–C(1) and N(2)–C(5) bond are elongated in both the cations **5A** and **5B** (Table 1), whereas the C(1)–C(2) and C(4)–C(5) bonds are shortened in both the cations in comparison with average bond lengths of pyridine[11] (Table 1).

In Table 1 the parameters of "quinonoid" deformation of pyridine ring are given in comparison[7] with those of 1-(N-methoxyamino)pyridinium perchlorate (**6**) with unsubstituted on 4-site as reference etalon and 1-(N-alkoxyamino)-4-dimethylaminopyridium salts (**3a**),[1] (**7**)[8] and (**8**)[8] (Scheme 3). The corresponding bond lengths of compounds **2a**, **3a**, **5A**, **5B**, **7** and **8** are similar in contrast to those in 4-unsubstituted **6**. Evidently, the nature of the substituent (H, CO_2Et, $C(O)NH_2$, $C(O)NMe_2$) at nitrogen atom, which are bound with alkoxy group, causes slight influence on "quinonoid" deformation of pyridine ring.

In **5** atom N(2) has planar configuration. The sum of bond angles centered at this nitrogen atom (∑β) is 359.9º as in **5A** as in **5B**. But amide nitrogen atom N(1), the central atom of geminal systems O-N-N^+, has pyramidal configuration. Its ∑β is equal 335.6º in (**5A**) and 335.4º in **5B**. Among anomeric amides[12,13] the existing in two or more forms differing by the pyramidality degree of the amide nitrogen atom is known for N-ethoxy-N-chlorourea[14] and N-chloro-N-methoxy-N'-(4-nitrophenyl)urea.[15] Also, N-[(benzo-yl)(hydroxyl)methyl]-N-benzyloxy-N'-(2-bromo-phenyl)-urea exists in two forms which vary in a different degree of pyramidality of the same nitrogen atom.[16]



Scheme 3. Structure of **6**, **7**, **8** and **9**.

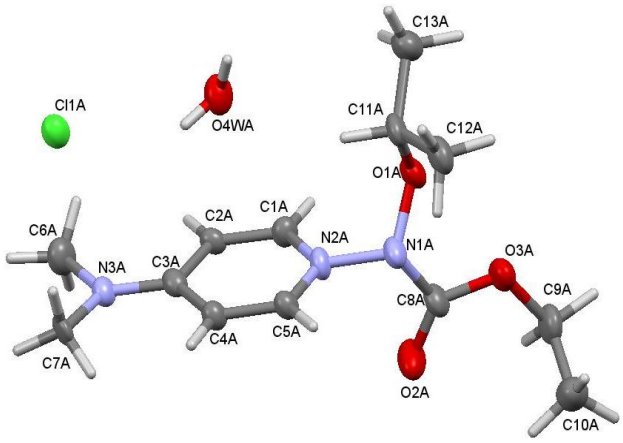


Figure 1. Molecular structure of **5A** monohydrate.

Table 1. Pyridine ring deformation in 1-(N-alkoxyamino) pyridinium salts (**3a**), (**5A**), (**5B**), (**6**), (**7**) and (**8**).

Compound	Bond lengths, Å			
	N2-C1, N2-C5	C1-C2, C4-C5	C2-C3, C3-C4	C3-NMe2
6[7]	1.341(2)	1.385(3)	1.349(5)	-
	1.341(2)	1.385(3)	1.387(4)	
5A	1.348(7)	1.360(7)	1.432(7)	1.337(7)
	1.368(7)	1.362(7)	1.411(8)	
5B	1.356(7)	1.352(7)	1.429(7)	1.326(7)
	1.349(8)	1.346(8)	1.427(8)	
3a	1.349(2)	1.351(2)	1.414(2)	1.337(2)
	1.354(2)	1.355(2)	1.416(2)	
7	1.361(2)	1.353(3)	1.425(2)	1.324(2)
	1.345(2)	1.341(3)	1.426(2)	
8	1.366(3)	1.349(3)	1.430(3)	1.333(3)
	1.346(3)	1.334(3)	1.413(3)	
Py	1.337	1.380	1.379	

Numbering of pyridine ring shown Figure 1 has been used

In compound **5** the ∑β is more in comparison to those in known 1-(N-alkoxyamino)pyridium salts (**3a**, **b**),[1] (**6**),[7] (**7**)[8] and (**8**)[8] (Table 1).

Probably it is caused by higher electron withdrawing capacity of EtO_2C-substituent compare to hydrogen (**3a**, **3b**), carbamoyl (**6**, **7**) and dimethylcarbamoyl (**8**) substituents. In anomeric amides the presence of electron withdrawing substituent at pyramidal amide nitrogen atom diminishes it pyramidality degree.[12,13]

The lone pair (Lp) of the N(1) atom is almost coplanar to the pyridine plane (the LpN(1)–N(1)-N(2)-C(1) torsion angle (TaLPPy) is -10.4º in cation **5A** and 14.6º in cation **5B**. The conjugation between LpN(1) and π-system of pyridine is impossible. The same type of LpN(1) orientation was revealed for other 1-(N-alkoxyamino)pyridium salts **3a**, 3**b**,[1] **6**,[7] **7**[8] and **8**[8] (Table 2).

In compound **5** the $N-N^+$ bond is elongated compare to the N–N bond in hydrazides of carboxylic acids (1.400 Å[17]). Probably, this $N-N^+$ bond elongation has been caused by $n_{O(Me)} \rightarrow \sigma^*_{N\text{-}N+}$ anomeric effect action as in cases of 1-(N-alkoxyamino)pyridium salts **3a**, **b**,[1] **6**,[7] 7,[8] and **8**.[8]

The lengths of the amide N–C bond in compound (**5**) is close to that in methyl N-(4-chlorobenzoyloxy)-N-methoxycarbamate **9** (1.423(2) Å[18]). The degrees of the nitrogen pyramidality in compounds **5** and **9** (∑β 334.1) are also close.[18] There is some elongation of the amide N–C bond in compound **5** compare to that in amides[19] (1.359 Å). It is caused by different degrees of C=O conjugation with sp^3 hybridized nitrogen atom in compounds **5**, **9** and sp^2 hybridized nitrogen atom in usual amides.[12,13,19]

In compound **5** ester moiety adopts +*sc*- and -*sc*-conformations relatively to the N(1)-N(2) bond, respectively. The C(11)–O(1)–N(1)–N(2) torsion angle is 77.8(5)º in **5A** and -81.4(5)º in **5B**. Ethoxy moiety has -*sc*-conformation toward to the C(8)–O(3) bond, the C(8)–O(3)–C(9)–C(10) torsion angle is -77.1(7)º in (**5A**) and -84.8(8)º in **5B**.

The isopropyloxy substituent is situated in *sp*-conformation toward to the N(1)–N(2) bond. The N(2)-N(1)-C(8)-O(2) torsion angle is-23.3(8)º in **5A** and 20.3(10)º in **5B**. This orientation of substituent is stabilized by attractive intramolecular shortening contact N(2)…H(11) 2.65 Å in **5A** and 2.63 Å in **5B**, the van der Waals radii sum is 2.66 Å.[20]

Table 2. Some structure parameters in 1-(N-alkoxyamino)pyridium salts

Compound	∑β, °	Bond lengths, Å			
		N−N+	N−OR	N−C(O)	TaLPP, °
5A	335.6	1.418(6)	1.411(6)	1.428(7)	-10.4
5B	335.4	1.421(6)	1.414(6)	1.419(8)	14.6
3a (ref.1)	312	1.428(2)	1.431(2)	-	4
3b (ref.1)	312	1.426(1)	1.440(1)	-	17
6 (ref.7)	333.9(3)	1.4254(18)	1.3999(17)	1.4515(19)	0.2
7 (ref.8)	332.7	1.413(2)	1.411(2)	1.450(2)	6
8 (ref.8)	324.22	1.425(3)	1.429(3)	1.465(3)	10.6

In crystal of **5** due to system of intramolecular hydrogen bonds with participating of chloride anions and bridging molecules of water three-dimensional network takes place:

O(4WA)−H(4WA)...Cl(1A)' (x,y,z) H…Cl' 2.50 Å, O−H….Cl' 142˚;

O(4WA)−H(4WB)...Cl(1A)' (2-x,-y,1-z) H…Cl' 2.46 Å, O−H….Cl' 157˚;

O(4WB)−H(4WC)...Cl(1B)' (x,y,z) H…Cl' 2.37 Å, O−H….Cl' 165˚;

O(4WB)−H(4WD)...Cl(1B)' (1-x,1-y,1-z) H…Cl' 2.58 Å, O−H….Cl' 143˚;

C(1A)−H(1A)...Cl(1B)' (1-x,1-y,1-z) H…Cl 2.90 Å, C−H…Cl 125˚;

C(1B)−H(1B)...Cl(1A)' (1-x,1-y,1-z)H…Cl 2.58 Å, C−H…Cl 143˚;

C(2A)−H(2A)...Cl(1B)' (1-x,1-y,1-z) H…Cl 2.87 Å, C−H…Cl 127˚;

C(4B)−H(4B)...Cl(1A)' (2-x,1-y,1-z) H…Cl 2.76 Å, C-H…Cl 134˚;

C(5A)−H(5A)...Cl(1B)' (2-x,1-y,1-z) H…Cl 2.68 Å, C−H…Cl 138˚;

C(7A)−H(7AC)...Cl(1A)' (x,y,z) H…Cl 2.81 Å, C−H…Cl 143˚;

C(7A)−H(7AC)...Cl(1A)' (x,y,z) H…Cl 2.81 Å, C−H…Cl 143˚;

C(7A)−H(7AB)...O(2A)' (2-x,1-y,1-z) H…O 2.40 Å, C−H…O 176˚;

C(7B)−H(7BC)...C(5A)' (π) (2-x,1-y,1-z) H…C 2.73 Å, C−H…C 148˚

Conclusion

The first stable 1-(N-alkoxy-N-alkoxycarbonyl)amino-4-dimethylaminopyridium salt, 1-(N-ethoxycarbonyl-N-isopropyloxy)amino-4-dimethylaminopyridinium chloride, has been synthesized. XRD study of it structure has been done.

Acknowledgements

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SYNTHESIS OF SCHIFF BASE METAL COMPLEXES: A CONCISE REVIEW

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Keywords: Schiff base, metal ion, dentation, synthesis, coordination.

Schiff bases are a group of compounds prepared by the condensation of primary amines and active carbonyl compounds. The Schiff bases have a general structure RR′C=N-R″ (where R, R' and R″ are alkyl, cyclohexyl, hydroxyalky1, hydroxyary1, etc). Herein the different synthetic routes of Schiff Base complexes like, direct synthesis, in situ method, oxidation of coordinated secondary amine, amine exchange approach, metal exchange and ligand exchange reaction are reviewed. A concise survey of literature on the coordination modes of complexes of Schiff base ligands is also presented in this article. The coordination complexes have been successfully screened against different strains of bacteria where they depicted the potential antimicrobial behaviour.

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Introduction

The chemistry of coordination compounds is well known and widely studies, yet many new developments are continuously taking place in this area of inorganic chemistry. The preparative work of Jorgenson, Werner and many others during the turn of the century opened up new vistas in coordination chemistry of transition metals. It was Alfred Werner[1] who systematized the subject coordination chemistry, by propounding theory in 1893, for which he awarded Nobel Prize in 1913. These ideas have considerably enriched the understanding of the nature of metal-ligand bonds, structure and chemistry of metal complexes, their stabilities and liabilities and other properties. It is now the need of time that new ligands of specified design should be synthesized which could lead to metal complexes with special desired and possible predictable properties. The steady improvement in synthetic methodology allows us to foresee coordinating chemistry entering a phase of creative chemistry.

For a long time the coordination compounds were considered as a rare and special class but now they have been recognized as the most resourceful type of compounds which help the nature to convert simple inorganic molecules into organic matter. For instance CO_2 and H_2O are converted into sugars by coordination compound, called chlorophyll, the magnesium porphyrin complex present in plants. A similar iron porphyrin complex present in hemoglobin of animals operates in the red bloods cells as a carrier of oxygen for the oxidation of organic matter to produce carbon dioxide water and energy. Hemoglobin is also concerned with the transport of CO_2 and acid base balance of the body.

Many metallo-enzymes are involved in diverse metabolic pathways including DNA synthesis, sugar metabolism and protein modification.[2]

Coordination chemistry is now recognized as an independent discipline covering a wide range of areas from medicine to environment. In recent past there has been a great upsurge in the studies of metal complexes of bioinorganic medicinal relevance. The discovery and basic concepts of medicinal inorganic chemistry have recently reviewed.[3-5] The field now encompasses active metal complex, metal ions and even metal ions can be removed from a biological system by judicious use of metal binding molecules (termed ligands from the Latin word ligare, meaning that which binds).

Medicinal inorganic chemistry is at the interface between medicine and inorganic chemistry, and includes metal-based drugs, metal sequestering and mobilizing agents and metal containing diagnostic aids.[6,7] In the early systematic study of metals in medicine (during the early to middle part of the twentieth century), recognition of the essentiality of some metal ions (e.g. iron, zinc and copper) for the avoidance of deficiency disease was a major step forward. Not only some many metal ions are essential nutrients, but many are also becoming increasingly prevalent components of diagnostic or the therapeutic agents to study or treat wide variety of disease and metabolic disorders.[3,7,8] The list of metal ions that qualify for essential status is a work in progress; it includes not only expected members such as zinc, copper and manganese but also man formerly thought of only a poisonous, such as selenium and molybdenum.[9,10] Included in the "possibly essential" list are such unexpected candidates as arsenic, nickel, silicon, and vanadium.[10-12]

The applications of metal complex in qualitative and quantitative chemical analysis have also been the subject of numerous studies during the past decade.[13] To understand the level of pollution, analysis or measurement of the level of pollution is undertaken called the environment pollution analysis. This indicates the exact degree of pollution. Coordination compounds have found a very important place in environmental chemistry for determination of pollutants by complexometric and spectrophometic methods.

The development of complexes for application in medicine is an obvious example of investigation and creativity. Remarkable example is *cis*-platin, *cis*-$PtC1_2(NH_3)_2$ introduced by Rosenberg,[14] which is successfully employed world wide as anticancer drugs.[15,16] Use has been made of the calcium complexed EDTA as a scavenger for removing heavy metals like Pb(II), Cd(II) etc in case of such heavy metal poisoning in human. Antitumour activity of Cu(I) and Cu(II) complexes have a attracted much attention.[17] Another important use of metal complexes and chelating molecules is in the field of therapy. The use of gold complexes in the treatment of tuberculosis dates back to 1917 and many complexes of gold are known to be useful in the treatment of arthritis since 1927. In the course of study of a number of lipid soluble gold complexes for possible application of arthritis therapy, Winstock and coworkers[18] prepared an unusual complex of gold. Many drugs are known to be potentiated by complexation with Mn^+ ions. Thus, Fe(III) increases antibacterial activity of 8-hydroxyquionline significantly, antibercular activity of isonicotinic acid hydrazide is increased tenfold by Cu(II), carcinostatic activity of many drugs is enhanced by chelation.[19]

The insulin-like effect of vanadium salts in cells[20,21] and diabetic animals[22-28] is been known since the 1980s. Diabetic patients frequently have both abnormal glucose and lipid metabolism that can be normalized by treatment with insulin. Studies testing compounds in animal model systems [23-28] and in human beings[29-32] show that simple vanadium salts and vanadium complexes alleviate the symptoms of diabetes.

Coordination compounds involving Schiff bases

In 1864, Schiff[33] prepared a series of compounds by the condensation of primary amines and active carbonyl compounds and since then they are known as Schiff bases. The Schiff bases have a general structure RR′C=N-R″ (where R, R' and R″ are alkyl, cyclohexyl, hydroxyalky1, hydroxyary1, etc). The Schiff bases contain an azomethine group and have azomethine moiety (C=N). The donating properties of the lone pair of electron increases when a functional group such as -SH or -OH is sufficiently near to the azomethine moiety and this facilities the formation of a stable metal complex.

The coordination chemistry of the metal chelates of Schiff bases is known for more than a century. In 1840, Ettling[34] prepared the copper(II) complex with salicylideneimine. Since then a good amount of literature[35-52] on Schiff bases and their metal complexes have appeared.

Layer in 1963 revived the synthesis of Schiff bases and also studies their physical and chemical properties.[53] Dwyer and Mellor[54] described their chelating characteristics in a monograph. Holmes and co-workers[55] have presented an excellent review of the metal complexes of Schiff bases and Bayer indicated a novel approach to the structure and specificity of the organic chelating agents including some of the Schiff bases. Jungeis *et al*.[57] in 1969 have described the detailed applications of Schiff bases in inorganic analysis.

Synthetic routes to Schiff base complexes

Major development in the chemistry of transition metal complexes of Schiff bases took place in the last two decades and much emphasis had been laid on synthesis and structure. The synthesis, properties and structure of the Schiff base complexes depend upon nature of the Schiff base and metal ion. The following methods have generally been used for the synthesis of metal complexes of Schiff base.[58]

Direct synthesis

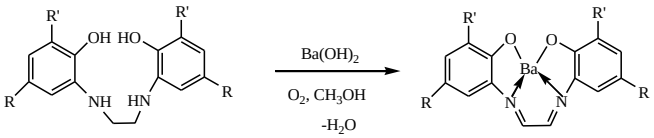
In this method, the Schiff base is allowed to react with metal ion in a suitable solvent. In order avoid the hydrolysis of azomethine group the use of organic solvent is preferred, although in a number of cases the use of binary azeotropic mixture of water and an organic solvent has been reported.

In situ method

In this method the metal ion is added during or shortly after the mixing of the aldehyde and amine. In a number of cases, it has been reported that the metal ion coordinates with one component and then reacts with other components, thus facilitating the reaction of complexation.

Oxidation of coordinated secondary amine

Here the metal ion facilities the oxidative dehydrogenation of the secondary amine and the Schiff base complexes are obtained directly.[59] One such reaction is given in scheme 1.



where R = H, n-C_4H_9, C_6H_5, R′ = C_4H_9, C_6H_5, *o*-HSC_6H_4

Scheme 1. Formation of Schiff base from secondary amine

Amine exchange reaction

In this method, an amine reacts with transition metal complex of Schiff bases and exchange of alky/aryl groups take place as illustrated below.[60]

$$Ni(o\text{-}OC_6H_4CH{=}NR)_2 + 2R'NH_2 \rightarrow Ni(o\text{-}OC_6H_4CH{=}NR')_2 + 2RNH_2 \quad (1)$$

where R = H, n-C_4H_9, C_4H_{10}, C_6H_5, *o*-HSC_6H_4

Metal exchange reaction

This method is used for the preparation of Schiff base complexes, which are difficult to obtain by any other method.

A typical example of metal exchange reaction is illustrated below.

$$CuCl_2 + TiSalen = Cu(Salen) + TiCl_2 \quad (2)$$

Ligand exchange reaction

This method is preferably used for the preparation of dioxomolybdenum(VI) complexes that are extremely difficult to synthesize by other methods. A typical reaction is given below.[61]

$$MoO_2(acac)_2 + SalenH_2 \rightarrow MoO_2(Salen) + 2acaeH \quad (3)$$

where acacH = acetylacetone

Coordination modes of Schiff base ligands/mixed ligands

It is well known that the Schiff base/mixed ligands coordinates as bi-, tri-, tetra-, penta-, hexa- and hepta-dentate ligands. A brief survey of literature on the coordination complexes of ligand is presented below.

Coordination complexes with bidentate Schiff base ligands / mixed ligands.

The Schiff base derived from aromatic *o*-hydroxyaldehyde and primary amine behave as bidentate ligands. Generally the stereochemistry of the bidentate Schiff base complexes depend upon the group present in the Schiff base on increasing the size of the R group the stereochemistry changes from square planar to tetrahedral. The Co(II) complexes of bidentate Schiff base (Figure 1) (R = H, OH) are square planar.

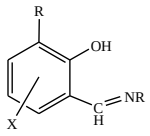


Figure 1. A bidentate Schiff base ligand.

Topich[62] has achieved synthesis of a heterochelate complex $MoO_2(Acac)L$ (where LH = Schiff base) by the reaction of $MoO_2(acac)_2$ and Schiff base (Figure 2) in ethanol or ethyl acetate.[63] Only one acetylacetone moiety was not displaced even when the metal to ligand ratio was greater than unity. IR data indicates that the ligand coordinates through to oxygen atom of the carboxylic acid group and nitrogen atom of azomethine group and not through the phenolic oxygen atom.

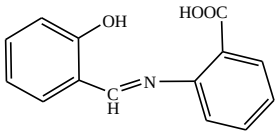


Figure 2. A Schiff's base ligand

Dioxomolybdenum(VI) complexes of the type cis-MoO_2L_2 have also been prepared with the Schiff bases derived from hydroxylamine and *o*-hydroxyacetophenone or 2,4-dihydroxyacetophenone.[64] Hill et al.[65] have described the synthesis of complex of the type MoO_2L_2 with the Schiff bases (Figure 3 and 4) by the reaction of bis(acetylacetonato)dioxomolybdenum (VI) and the Schiff bases in THF. The Schiff base (**4**) yielded a tetrameric complex of the type $Mo_4O_{11}L_2$. The ligands in complexes of the type cis-MoO_2(acac)L are (**5**) and (**6**). Although the ligands are potentially tridentate, they behave as OH donor bidentate monobasic and NS donor bidentate monobasic ligands in cis-MoO_2L_2 and only signal due to the azomethine proton in the NMR spectra of dioxomolybdenum (VI) complexes (**3**) and (**5**) confirms the presence of a *cis*-MoO_2 structure.

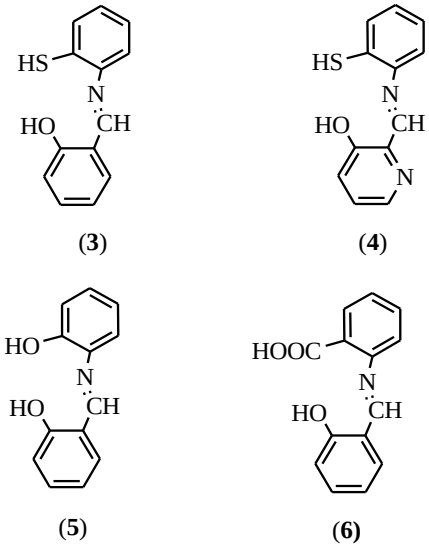


Figure 3-6. Schiff's bases forming complexes with dioxomolybdenum (VI).

Synthesis of two series of metal chelates[66] viz., (i) mononuclear chelates of composition $[VO(L_1)_2]H_2O$ and $[WO_2(SCN)C_2H_5OH)_2(L_1)]$ (where L_1H= N-(4'-butyrylidene-3'-methyl-1′-phenyl-2′-pyrazolin-5-one)-*p*-anisidine, 9BUMPHP-AH), N-(4-butyrylidene-3′-methyl-1′-phenyl-2′-pyrazolin-5′-one)-*m*-toluidine (BUMPHP-MTH), N-4′-butyrylidene-3′-methyl-1′-phenyl-2′-pyrazolin-5′-one)-*o*-phenylenediamine (BUMPHP-OPHDH$_2$), N,N′-bis-(4'-butyrylidene-3'-methyl-1'-pheny1-2'-pyrazolin-5'-one)-*m*-phenylenediamine (BMPH-MPHDH$_2$), or $VO(L_2')(H_2O]$ (where $L_2'H_2$=N,N'-bis(4'-butyrylidene-3'-methy1-1′-pheny1-2′-pyrazolin-5'-one)-m-phenylenediamine (BMPHP -MPHDH$_2$), and binuclear chelates of composition, $[[VO(H_2O)(OHO]_2L_2]$ and $[[WO_2(SCN)(H_2O)]_2(L_2)]$, where L_2H_2=N,N′-bis(4′-butyrylidene-3′-methyl-1′-pheny1-2′-pyrazolin-5'-one(-*p*-phenylenediamine (BMPH-PPHDH2) or N,N'-bis(4′-butyrylidene-3′-methyl-1′-pheny1-2′-pyrazolin-5′-one)benzidine (BMPHP–BZh$_2$) have been reported.[67]

Six new binuclear dioxomolybdenum(VI) complexes or the composition $[\{MoO_2(O_{11})_2\}_2(L)_{21}$, where L=N-(4'-benzoylidene-3'-methyl-1'-pheny1-2'-pyrazoline-5'-one)sulphamethoxazole (BMPHP-SMZ, N-(4'-benzoylidene-3'-

methyl-1'-pheny1-2'-pyrazoline-5-one)sulphadimidine (BMPHP-SIAM), N-(4'-benzoylidene-3-methy1-1-phenyl-2'-pyrazoline-5'-one)sulphadiazine (BMPHF-SDZ), N-(4'-benzoylidene-3'-methy1-1'-pheny1-2'-pyrazo1ine-5'-one)-sulphanilamide (BMPHP-SNM), N-(4'-benzoylidene-3'-methyl-1'-phenyl-2'-pyrazolin-5'-one)sulphamerazine (BWIPT-TP-STVIR) or benzoylidenethy1-1'-pheny1-2'-pyrazolin-5'-one)sulphadimethoxine (BMPHP-SMX) have recently been reported by Maurya et al.[67] These complexes are reported to be obtained by the interaction of bis(acetylacetonato)dioxomolybdenum(VI) with the said sulpha drug based ligands in ethanol.

A new series of novel mixed-ligand complexes of Cu(II), Ni(II), Co(II), Zn(II), Sm(III) and U(VI)O_2 with the Schiff base derived from salicylaldehyde and the sulfadrug sulfamerazine, [N-(salicylidene)sulfamerazine] (LH) (Figure 7) and the heterocyclic base, 2,2'-bipyridine (bpy) have been synthesized and characterized by Maurya et al.[68] through IR, NMR, diffuse reflectance spectra and magnetic, thermal, and molar conductance measurements. The coordination by the azomethine nitrogen is inferred by the down field shirting or the -CH=N- signal in the NMR spectra and the shift of v(C=N) to lower frequencies by 1540 cm^{-1} in the IR spectra upon complexation. Conductance measurements confirm the non-electrolytic nature of these complexes. The presence or lattice and coordinated water molecules are indicated by thermograms of the complexes. The general compositions of the complexes were found to be [M(L)(bpy)(OAc)(H_2O)], [Sm(L)(bpy)$(OAc)_2(H_2O)]_2H_2O$ and [UO_2(L)(bpy) (OAc)(H_2O)H_2O, where M=Cu(II), Ni(II), Co(II) or Zn(II) and HOAc=acetic acid.

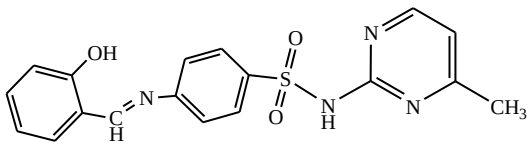


Figure 7. [N-(salicylidene)sulfamerazine] (LH).

Thompson et al.[69] have reported bis(3-hydroxy-4-pyranato)oxovanadium(IV) and tris(3-hydroxy-4-pyrone)vanadium(III) complexes, assessed them for insulin mimetic potential.[70] Key feature of these ligands are a six member ring, with a ring N or O atom either ortho or para to ketone group, and hydroxypyridinones are characterized by synthetic versatility and high affinity for a range of metal ions, rendering these ligands excellent choices for the formulation of therapeutic and/or diagnostic metallopharmaceuticals.

A series of bis(maltolato)oxoyanadium(IV) (BMOV), bis(maltolato)dioxomolybdenum(VI), bis(maltolato)chromium(III), bis(maltolato)copper(II) and bis(maltolato)cobalt(II) complexes have recently reported by Thornpson et al.[71]

In view of insulin mimetic properties of oxovanadiurn(IV), Mohammadi et a1.[72] have synthesized novel bis(4-hydroxy-3-methoxyphenyI)-1,6-heptadiene-3,5-dione (curcumin) complexes with the formula, ML_3, where M is Ga(III) or In(III), or of the formula, ML_2 where M is $[VO]^{2+}$ and characterized by mass spectrometry, infrared absorption spectroscopy and elemental analysis. The ligand curcumin behave as monobasic bidendate in these complexes.

Four new solid derivatives, [V(IV)O(1,2-diethy1-3-hydroxyl–4-pyridonate)$_2$], (V(IV)O(1-(p-toly1)-2-ethy1-3-hydroxyl-4-pyridonato)2,], [V(IV)O(1-P-(n-buty1)pheny1)-2-ethy1-4 pyridiononato)$_2$] and (V(IV) O(1-hexyl) phenyl-2-ethy1-3-hydroxyl-4-pyridonato)$_2$], were isolated and characterized by Garribba et al.[73] Aqueous solution studies regarding the identification and characterization of complexes formed by the V(IV)O ion and 3-hydroxyl-4-pyridone derivatives have been performed using EPR and UV-Vis spectroscopic techniques. Garribba et al.[74] have recently reported 2-pyrazinecarboxylic acid and three of its derivatives (5-methyl-2-pyrazinecarboxylic, 2,3-pyrazinedicarboxylic and 5-hydroxypyrazinecarboxylic acids) coordinate the V(IV)O ion forming VOL, VOL_2 and $(VO)_2L_2H_2$ species in acidic and neutral solutions. Bis chelated species are hexacoordinated and are characterized by a cis/trans isomerism, with the trans arrangement favoured with respect to the cis one. The ligand used in this synthesis behave as monobasic bidentate with an N,O coordination are characterized by an ($N_{aromatic}$, COO^-) donor set.

Coordination complexes with tridentate Schiff base ligands/mixed ligands

The tridentate Schiff bases are obtained by the condensation of amines containing OH or SH group and o-anisidine-hydroxycarbonyl compounds or β-diketones. The ligand (Figure 8) derived from salicylaldehyde and *o*-aminophenol is an important example of tridentate Schiff base. The tridentate Schiff base ligands react with the metal ions to give metal complexes having novel structural, magnetic and spectroscopic properties.[75-77]

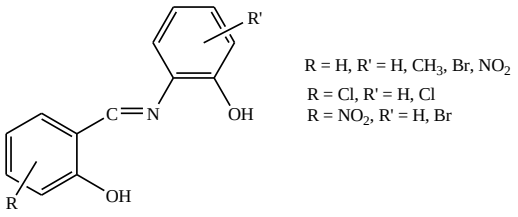


Figure 8. Substituted (*E*)-2-(hydroxybenzylideneamino)phenols.

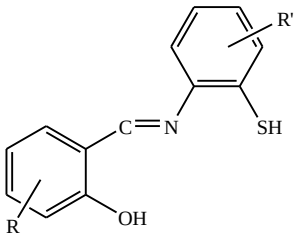


Figure 9. Schiff' base forming complex with oxovanadium(IV).

The oxovanadium(IV) complexes of the type VOL (Figure 9, Substituent(s) = 5-chloro; 5-chloro, 5-bromo, 5-nitro, 3,5-dichloro, 5,6-benzo) have been reported by Lee et al.[78] The magnetic moments of these complexes are in the range 1.27-

1.32 B.M. The magnetic moments decrease considerably with lowering of temperature indicating the presence antiferromagnetic exchange. The *v*(V=O) stretch of the complexes lies in the range 900-1005 cm^{-1}. The electronic spectra of the complexes exhibit a broad band in the region 13,300-20,000 cm^{-1} due to the d-d transition. It has been noticed that oxovanadiuin(IV) complexes with ONS donor sites exhibit a greater degree of antiferromagnetism than the oxovanadium (IV) complexes with ONO donor sites.

A series of copper(II) and zinc(II) complexes involving a tridentate O,N,O-donor[79] Schiff base derived from salicylaldehyde and β-alanine {i.e. N-salicylidene-β-alanine(2-), L} having the composition $[Cu_2(L_2)H_2O)].H_2O$, $[Cu(L)(H_2O)]_n$ and $[Zn(L)(H_2O)]_n$ (**3**) have been prepared and characterized by elemental analyses. UV-Vis, FT-IR and electron spray ionization mass (ESI-MS) spectra and thermal analyses. Complexes (**1**) and (**2**) have been investigated by single X-ray analysis and also by temperature dependent magnetic susceptibility measurements (294-80 K). All complexes have been evaluated by the antiperoxynitrite activity assay and alloxan-induced diabetes model. The significant antioxidant activities have been found in the case of both Cu(II) complexes. In spite of this first two complexes, the Zn(II) complex, as well as the potassium salt of the ligand (KHL) showed only insignificant protective effect against the tyrosine nitration *in vitro.*

Coordination complexes with tetradentate bases/mixed ligands.

The tetradentate Schiff bases arc obtained by the condensation of an aromatic o-hydroxycarbonyl compound and a diamine. The important examples of the quadridentate Schiff bases (Figure 10) are the condensation products of salicylaldehyde or substituted salicylaldehyde and diamines. These Schiff bases enforce a high degree of planarity to the metal chelates.[80] With the change of $_n$ from 2 to 4, the stereochemistry of the Cu(II) and Co(II) complexes of Schiff bases changes from square planer to tetrahedral.[81-83]

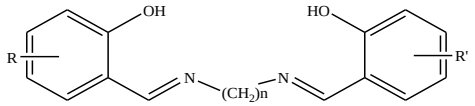
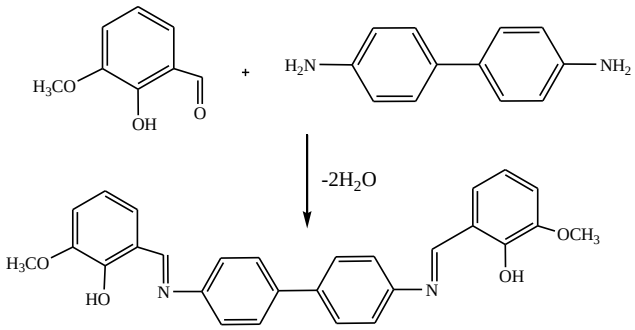


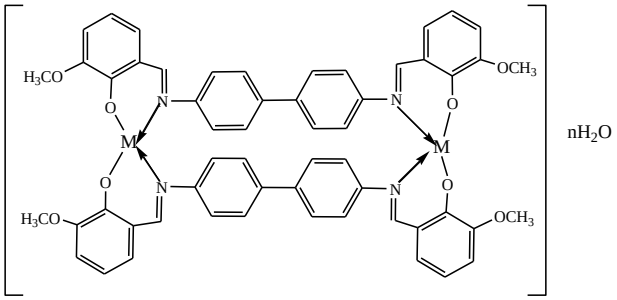
Figure 10. A tetradentate Schiff base ligand.

A binucleating tetradentate Schiff base ligand, bis (*o*-vanillin)benzidine (o-v_2bzH_2), and its seven new binuclear complexes have been synthesized (Scheme 2) and characterized on the basis of elemental analysis, IR, NMR, electronic, magnetic, thermal studies and conductance measurements. The compositions of these complexes were found to be $[M(o\text{-}v_2bz)_2.nH_2O$, where M = Cu(II), Ni(II), Co(ID, Zn(II), Mn(II) or UO_2(V1), and $\{Sm(o\text{-}v_2bz)(OAc)(H_2O)]_2$.

The ^{1}HNMR spectrum of one of the compounds, $[Zn(o\text{-}v_2bz)]_2$ shows the absence of proton signal for phenolic oxygen (-OH). Low magnetic moment values, high thermal stability and insolubility in common organic solvents support the binuclear structure of these complexes. Suitable binuclear structures (Figure 11) have been assigned. The 3D molecular modeling (Fig. 14) and analysis for bond lengths and bond angles have also been carried out of one of the representative compounds, $[Ni(o\text{-}v_2bz)_2]$ (2).



Scheme 2. Synthesis of bis(*o*-vanillin)benzidine.



M = Cu(II), n= 0, 2; Co(II), n = 0; Zn(II), n = 0; Mn(II), n = 0

Figure 11. Structure of binuclear complexes of a tetradentate Schiff's base.

Coordination Complexes with Penta-, Hexa- and Heptadentate Schiff Base/ Mixed Ligands.

Relatively few reports on the metal complexes of the Schiff bases having potential penta-, hexa- and heptadonor sites have appeared as compared to the metal complexes with bi-, tri- and tetradenate Schiff bases. The Schiff base (Figure 12) derived from diethylenetriamine and salicyladehyde behaves as a dibasic pentadentate (N_3O_2) donor's ligand.

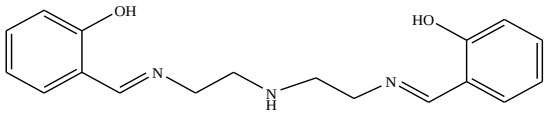


Figure 12. A dibasic pentadentate Schiff's base.

The coordination of pentadendate ligand to the central metal ion can make place only when the fifth axial donor atom present is flexible. The five-coordinated compounds can acquire two different geometries depending upon the flexibility of chelate ring and length of chain between the two coordinated atoms. Thus, the metal complex would have a trigonal bipyramidal geometry if the chelate ring is quite flexible and if the length of chain is small the resulting complex will be of square pyramidal geometry.

Dioxouranium(VI) complex of the type UO_2L (where LH_2=Figure 12) has been prepared by the reaction of an equimolar mixture of dioxouranium(VI) acetate and Schiff base.[85] The Schiff base behaves as a neutral pentadentate ligand. UO_2X_2 and $UO_2(ClO_4)_2$ react with the Schiff base shown in Figure 12 as a result nine coordinated complexes $UO_2(LH_2)X_2$ and UO_2L (where X=Cl^-, I^-, NO_3^- and $0.5SO_4^{2-}$) are formed, respective. The Schiff base behaves as a neutral pentadentate in $UO_2(LH_2)X_2$ and dibasic pentadentate ligand in UO_2L. The nitrate ion behaves as a monodentate ligand while the sulphate ion behaves as a bidentate ligand.

Dioxouranium(VI) complexes of the type UO_2L (where LH_2=Schiff base derived from salicylaldehyde and 1,5-diamino-3-oxopentane) have been reported.[84] The complexes exist in α- and β-forms. In both the modifications uranium is pentagonal bipyramidal with the pentadentate ligand coordinated in a plane normal to UO_2. These are conformational isomers differing mainly in the spatial arrangement of the ethylenic chains. The UO_2 group is non-linear in both the forms.

Dioxouranium(VI) complex of the type $[UO_2(LH_2)(NO_3)_2]_2^-$ $[UO_2(NO_3)_4]$ (where LH_2=Schiff base 2,6-diaeetylpyridine bis(2'-pyridylhydrazone) has been prepared by the reaction of uranyl nitrate and the Schiff base.[87] The Schiff base behaves as a neutral pentadentate ligand. IR data indicate the monodentate behavior of nitrate group in the cationic moiety while the bidenate behavior of two nitrate group and the remaining two as monodentate in the anionic part giving a six fold coordination of oxygen atoms in the equatorial plane normal to uranyl group.

The Schiff bases (LH_2) derived from substituted salicylaldehydes and bis(3-aminopropyl)amine or bis(3-aminopropyl)methylatninc from high-spin five coordinated complexes ML with manganese(II), cobalt(II), nickel(II) copper(II) and zinc(II).[88] The Schiff bases behave as dibasic pentadentate ligands. The nickel(II) complex when dissolved in pyridine forms hexacoordinated pyridine adducts.

Ten-coordinated dioxouranium(VI) complex, $UO_2(LH_4)X_2$ (where LH_4 = Schiff base prepared by the condensation of salicylaldehyde and triethylenetetraamine, X=$C1^-$, I^-, NCS^-, $0.5SO_4^-$) has been reported.[89] IR data indicate that the Schiff base function; as neutral hexadentate (N_4O_2 donors) ligand. The nitrate and thiocyanate groups behave as monodentate ligands while the sulphate behaves as a bidentate ligand.

A pentadentate Schiff base, 2,6,10-thiazo-1,11-bis(2-aminophenyl)undeca-1,10-diene (L) and complexes of general formula MLX_2 (where M=Cu(II), Ni(II), X = Cl, Br, I, NO_3^- and $C1O_4^-$) have been reported.[90] IR data show an interaction between halide anion of the outer coordination sphere and the complexes amino group. ESR and electronic spectral data of the copper(II) compound are consistent with a square pyramidal geometry. Since crystal ESR studies of $CuL(NO_3)_2$ and $CuLBr_2$ revealed that the copper atoms in the former compound occupy two magnetically non-equivalent places in the lattice while the copper atoms in the later compound take identical sites.

Synthesis and characterization of pentagonal bipyramidal complexes of nickel(II), cobalt(II), iron(II), manganese(II) and zinc(II) with the heptadentate base derived from 2,6-diacetylpyridine and diethylenetriamine have been reported.[91] The complexes are of the type $[ML]X_2.YH_2O$ (where M=Co(II), Ni(II), Fe(II), Mn(II), Zn(II), X=$C1O_4^-$, BPh_4, Y=0 or 1). A single crystal X-ray analysis of $[CuL](C1O_4)_2$ confirms the pentagonal bipyramidal structure.

A new mononuclear copper(II) complex $[Cu(HL)(Cl)PF_6$ having a donor[92] ligand (HL=N,N'-bis(2-pyridylinethyl)-1,3-diaminopropane-2-ol) has been synthesized and structurally characterized by X-ray crystallography. It shows a 1D chain in packing structure through intermolecular O-H…C1 strong hydrogen bonding interaction {O-H...C1, 1.99(4) Å and the angle (O-H...C1) 163(4)°}. The packing structure also shows π–π stacking. The morphology of sub micrometer rod of the complex has been studied by SEM.

Raman et al.[93] reported the synthesis, characterization and electrochemical behaviour of Cu(II), Co(II), Ni(II) and Zn(II) complexes derived from acetylacetone and *p*-anisidine and studied their antimicrobial activity. They found that neutral tetradentate N_2O_2 type complexes of Cu(II), Ni(II), Co(II) and Zn(II) synthesized using the Schiff base formed by the condensation of acetylacetone and *p*-anisidine possess square-planar geometry. All the title complexes were screened for antimicrobial activity by the well diffusion technique using DMSO as solvent. The minimum inhibitory concentration (MIC) values were calculated at 37 °C for a period of 24 h. It was found that all the complexes are antimierobially active and show higher activity than the free ligand.

Imran et al.[94] studied the *in vitro* antibacterial studies of ciprofloxacin-imines and their complexes with Cu(II), Ni(II), Co(II) and Zn(II). These were synthesized and characterized on the basis of physical properties, conductance measurements, elemental analysis, UV-vis, infrared and nuclear magnetic resonance spectroscopy. These ligands as well as their metal complexes were also evaluated for their antibacterial activity against several bacterial strains, such as *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella typhae*, and *E. coli*. They found that metal complexes are more antibacterial as compared to uncomplexed ligands.

Nida et al.[95] studied the synthesis, characterization and anti-bacterial studies of some metal complexes of Schiff base derived from benzaldehyde and sulfonamide. The research group subjected the complexes for their antimicrobial activity against *E. Coli* and *Salmonella Typhae*. The metal complexes of Cu(II), Ni(11) and Co(II) were found to show enhanced antimicrobial activity as compared to uncomplexed ligand.

Kavitha et al.[96] reviewed biological activities of schiff base and its complexes very efficiently. The discussion involves the mechanism and importance of these compounds. This review summarizes the synthesis and biological activities of Schiff bases and its complexes, Schiff bases are versatile ligands which are synthesized from the condensation of primary amines with carbonyl groups. These compounds are very important in medicinal and pharmaceutical fields because of their wide spectrum of biological activities. Most of them show biological activities such as antibacterial, antifungal as well as antitumor activity. Transition metal complexes derived from the Schiff base ligands with biological activity have been widely studied.

Singh et al.[97] surveyed synthesis and bioactivity of some metallo-sulpha drugs as per reports of that era. They summarized that compounds containing the sulphonamide group have long been used as drugs for various diseases. The biological activity of these drugs is enhanced on undergoing complexation with metal ions.

Sahu et al.[98] also recently reviewed the summary of medicinal chemistry features of schiff base and its enhancement of biological activities on complexation.

Donde et al.[99] reported the synthesis, characterization and biological activity of mixed ligand Co(II) complexes of schiff base 2-amino-4-nitrophenol-N-salicylidene with some amino acids. The Schiff base and its mixed ligand complexes, in general, were non-hygroscopic and stable solids. The compounds were subjected to simultaneous thermogravimetric analysis to study their decomposition mechanism and thermal stability. The Schiff base and mixed ligand complexes were preliminary screened against various strains of microbes to study their biological effect.

Tharinamj et al.[100] brought to light the synthesis and spectral characterization of some transition metal complexes of azomethine derivative of diaminomalononitrile. New complexes of VO(II), Mn(II), Co(Il), Ni(II), Cu(II), and Zn(II) were synthesized and characterized by analytical and physicochemical techniques. These metal complexes were also tested for their antibacterial and antifungal activities to assess their inhibiting potential. Metal-mediated fluorescence enhancement is observed on complexation of the azo Schiff base ligand. The synthesized compounds were investigated for nonlinear optical properties, and the surface morphology of the Cu(II) complex was studied by scanning electron microscopy.

Complexes of Co(II), Ni(II) and Zn(II) with Schiff bases derived from 4-anisaldehyde were reported by Ndahi and Nasiru 2012.[101] The electronic spectral data reported by them indicates that the compounds are six coordinated. The molar conductance values showed that the complexes are non-electrolytes, The compounds were screened in vitro for antibacterial activity against some pathogenic bacteria: *Escherichia Coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* using the agar-well diffusion method. The synthesized Schiff base complexes exhibit higher antibacterial activity against the tested pathogens compared to the free Schiff base because of chelation.

Antibacterial studies of some Schiff base metal complexes containing Zn(II), Cu(II), Ni(II), Co(II), Mn(II), Cr(III) and Cd(II) with some novel antibiotics have recently reported by Bukhari *et al*.[102] The antibacterial activity showed the following trend: Metal complexes > Schiff base ligands> Parent drugs.

Mahendra et al.[103] carried out synthesis, characterization and biological activities of 5-chloroisatin Schiff base and its metal complexes comprising of copper(II), cobalt(II), nickel(II) and zinc(II). The ligand and its metal complexes have been screened for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, etc. and antifungal activity against *Aspergillus niger*, *Aspergillus flavous*, etc. The activities of both the samples have shown significant and noticeable changes on complexation.

Transition metal complexes with mixed nitrogen-sulphur donor macrocyclic schiff base ligand of their synthesis, spectral, electrochemical and antimicrobial studies were recently updated by Shiekh et al.[104] with Cu(II), Co(II), Ni(II) and Mn(II). The results indicate that the complexes are having potential antibacterial and antifungal properties.

Besides experimental studies in the above systems computational aspects are very well studied. As per the application assets of the field various measures are guessed using theoretical field. Recently Ahmad et al.[105] reported inhibition effects of a synthesized novel 4-aminoantipyrine derivative on the corrosion of mild steel in hydrochloric acid solution together with quantum chemical studies, electronic properties such as highest occupied molecular orbital energy (HOMO), lowest unoccupied molecular orbital energy (LUMO) and dipole moment (μ) were calculated and discussed. The results showed that the corrosion inhibition efficiency increased with an increase in the HOMO values but with a decrease in the LUMO value.

Sulfa drug based complexes have been recently also studied with so many transition metals. Sharma *et al*. [106] brought to light the coordination chemistry of Ca(II) and Mn(II) with Schiff base of sulfaguanidine [4-amino-N-{amino(imino)methyl}benzenesulfonamide' and salicyl-aldehyde. The ligand behaves as a bidentate with N,O donor atoms. Complexes have been characterized by elemental analysis, UV-visible and IR spectral studies.

Conclusion

A concise survey of literature on the coordination modes of complexes of Schiff base ligands is also presented in this review article. It is clear from this review article that the Schiff bases coordinate as bi-, tri-, tetra-, penta-, hexa- and heptadentate ligands to the metal ions. The five-coordinated compounds can acquire two different geometries depending upon the flexibility of chelate ring and length of chain between the two coordinated atoms. The coordination complexes have been successfully screened against different strains of bacteria where they depicted the potential antimicrobial behavior.

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