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Synthesis of a new dithiocarbamate cobalt complex and its nanoparticles with the study of their biological properties

H. Nabipour[1,2,*]

[1]*Department of Chemistry, Islamic azad university, Takestan branch , Takestan, Iran*
[2]*Member of young researchers club, Islamic azad university, Takestan, Iran*

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Abstract

Nano dithiocarbamate (DTC) complex have been prepared by reaction between dithiocarbamate and metal salt under ultrasound irradiation. Reaction conditions such as the ligand DTC and phennantroline concentration, aging time and the ultrasonic device power show important roles in the size, morphology and growth process of the final products. The dithiocarbamate complex nanoparticles have been prepared by water solvent. The ultrasonic treatment applied for preparation nanoparticles. The antibacterial activity of nanoparticles derivatives tested against microorganism and compared with non-nano conditions. The resulting nanoparticles were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

Keywords: *Nanoparticles, Antibacterial activity, Characterization, Dithiocarbamate*

1. Introduction

Dithiocarbamates are versatile ligands capable of forming complexes with most of the elements and able to stabilize transition metals in a variety of oxidation states [1]. This property of stabilizing high oxidation states in metal complexes reflects strong o-bonding characteristic of these ligands.

Although the sulphur atoms of dithiocarbamato ligands possess o-donor and n-back-donation characteristics of the same order of magnitude, these ligands have a special feature in that there is an additional n-electron flow from nitrogen to sulphur *via* a planar delocalized π- orbital system, as shown below:

* Corresponding author: Hafezeh Nabipour
Islamic Azad University, Takestan Branch,
Department of Chemistry, Takestan, Iran
Tel + 98 9127890813, Fax +9828205270145
Email *Ha.nabipour@gmail.com*

This effect results in strong electron donation and hence a high electron density on the metal leading to its next higher oxidation state [2]. While dithiocarbamate complexes have been known for over a century, with many thousands having been prepared, the vast majority of these contain only simple alkyl substituents such as methyl and ethyl. A developing interest in the area of dithiocarbamate chemistry is the functionalization of the backbone such that new applications and interactions can be developed. This area is still in its early stages but already interesting potential applications have been noted including the functionalization of gold nanoparticles, the stepwise build-up of multimetallic arrays, the synthesis of dithiocarbamate-containing supramolecular systems which can be used for anion binding, the development of technetium radiopharmaceuticals [1]. Dithiocarbamates are a class of metal-chelating, antioxidant compounds with various applications in medicine for the treatment of bacterial and fungal infections, and possible treatment of AIDS [3].

In this view, we synthesized cobalte (II) complex with 1,10-phenantroline and a sulfur donor ligand such as pentamethylen dithiocarbamate sodium salt. The ultrasonic treatment used for preparation nanoparticles of dithiocarbamate. We have synthesized dithiocarbamate complexes and characterized by elemental analyses, IR, UV-Visible, ^{1}H NMR and ^{13}C NMR spectroscopic studies. The antibacterial activities of synthesized compounds were studied against two Gram-negative species, *Escherichia coli, Klebsiella pneumoniae* and two Gram-positive species, *Staphylococcus aureus* and *Bacillus subtilis* and, for *in vitro* antifungal activity against, *Candida albicans, Aspergillus flavus, Aspergillus nigar*.

2. Materials and methods

The reagents and solvents were of analytical grade. 1,10- Phenanthroline, amine, carbon disulfide were purchased from Merck Company. ^{1}H and ^{13}C NMR measurments were recorded on a Bruker 300 spectrometer (300 and 75 MH, respectively) in $CDCl_3$ using TMS as the internal reference. IR spectra of the compounds as KBr-disks were recorded in the range of 400 – 4000 cm^{-1} with a Mattson 1000 FT spectrometer. Melting points of sulfonamide derivatives were determined on a Gallenkamp melting point apparatus and are uncorrected Powder X-ray diffraction (XRD) was carried out on a Philips diffractometer of X'pert Company with monochromatized Cu Kα radiation. A multiwave ultrasonic generator (Sonicator-3000; Misonix, Inc., Farmingdale, NY, USA), equipped with a converter/ transducer and titanium oscillator (horn), 12.5 mm in diameter, operating at 20 kHz with a maximum power output of 600 W. The microdilution broth method was used to determine the antibacterial activity of compounds against the bacteria: *S. aureus* ATCC 25923, *B. subtilis* ATCC 1023, and *K. pneumoniae* ATCC 10031, *E. coli* ATCC 8739, *C. albicans* ATCC 10231, *A. flavus* ATCC 9170, *A. nigar* ATCC 16404.

2.1. Synthesis of pentamethylene dithiocarbamate

To a stirred solution of piperidine (0.05 mol) in chloroform (5 ml) (was added), at less than 4°C, carbon disulfide (3 ml, 0.05 mol) and sodium hydroxide (50% aqueous solution, 4 ml) was added. After stirring for 2 h, evaporation of volatiles was performed without heating. The obtained

precipitates were filtered, washed with ether, recrystallized from acetone and dried in vacumm over P_2O_5. Yield: 92%, m.p. 160 °C. Anal. Calc. for $C_6H_{11}NS_2$ (Mw = 161): C: 44.72; H: 6.83; N: 8.69; S: 39.75 Found: C, 44.70; H, 6.81; N: 8.67; S: 39. 71 %. IR (KBr) (cm^{-1}): 2939-2849 (m), 2526(w), 1488(w), 1440(s), 835 (s), 1060 (s). ^1H NMR ($CDCl_3$,): δ (ppm) 1.39-1.67 (m, 10H), 2.00 (s, 1H).

2.2. Synthesis of complex [Co (pipdtc)$_2$ (1,10-phen)]

An H_2O solution $CoCl_2.6H_2O$ (1 mmol) and an H_2O solution of the 1,10-phenanthroline (1 mmol) were mixed with stirring. The pentamethylene dithiocarbamate (2 mmol) ligand was then added dropwise with vigorous shaking. Dark green complexes separated out, which were filtered, washed thoroughly with H_2O recrystallized from CH_3Cl and dried *in vacuo* over P_2O_5. Yield: 85%, m.p. 220 C°. Anal. Calc. for $C_{24}H_{28}CoN_4S_4$ (Mw = 560.70): C, 51.50; H, 5.03; N, 9.99; S, 22.87. Found: C, 51.52; H, 5.05; N, 10.02; S, 22.88 %. IR (KBr) (cm^{-1}): 2935.83, 2853.46, 1620.85, 1470.36, 1515.48, 1359.88, 1237.28, 976.44, 887.24, 612.36, 400.07, 350.36. ^{13}C NMR ($CDCl_3$, TMS, 75.45 MHz): δ (ppm) 25.62, 26.98, 51.89, 121.37, 126.09, 128.40, 134.99, 134.99, 145.28, 149.31, 200.1. Molar conductance measurement for the complex is 50.36 $\Omega^{-1}mol^{-1}cm^2$.

2.3. Synthesis of dithiocarbamate nanoparticles

Ultrasonic device was employed to improve the dispersibility of the complex dithiocarbamate nanoparticles dispersed in aqueous solutions. To prepare the complex dithiocarbamate precursor amount of 1,10-phenantroline solution with concentrations of 1M was added to the 2M solution of pentamethylene dithiocarbamate in water. Therefore 1M solution $CoCl_2.6H_2O$ was added to the solution. Then the suspension was ultrasonically irradiated with a high-density ultrasonic probe immersed directly into the solution. The obtained suspension was allowed to age for 60 min. The precipitate was separated from mother liquor by using a centrifuge at 4000 rpm for 1 min, and at least 2 cycles of washing (using 25 °C deionised water) and centrifuging were required to removal of the residual impurities. The final product was dried at 50 °C in a vacuum system. The working parameters of the ultrasonic device were 60 kHz and 40 W/cm^2.

2.4. Characterization of nanoparticles

X-ray diffraction (XRD) technique was used to determine the ingredients of the sample. The morphology of nanoparticles was observed using a scanning electron microscopy (SEM).

2.5. In vitro antifungal activity

The compounds have been screened in vitro against *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. Among several methods [4] available, [5,6] microbroth dilution is common in use in recent times has been adopted.

2.6. Microbroth dilution assay

The susceptibility of the fungi to various fractions of compounds was assayed by microbroth dilution method. Sabouraud dextrose medium was dissolved in glass double distilled water and autoclaved at 10 psi for 15 min. A volume of 90 µL of medium was added to the wells of cell culture plates (Nunc Nunclon). The different concentrations in the range of 50 - 400 µg/mL of various fractions were prepared in duplicate wells and then the wells were incubated with 10 µL of conidial suspension containing 1×10^4 conidia. The plates were incubated at 37 °C and examined

macroscopically after 48 h for the growth of *Aspergillus* mycelia. All experiments were carried out in duplicate and the results were confirmed in three independent experiments.

2.7. In vitro antibacterial activity

The compounds have been screened in vitro against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus , Bacillus subtilis.* Various methods [7-10] are available for the evaluation of the antibacterial activity of different types of complex. However, the most widely used method [10] consists in determining the antibacterial activity of the complex is to add it in known concentrations to the cultures of the test organisms.

2.7.1. Disc diffusion assay

Method of paper disc diffusion 0.05 mol/L aqueous solution of nano complex was prepared, and the antibacterial activity of the complex against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* and *Bacillus subtilis* was studied. The bacterium suspension concentration was controlled as 5×10^5–5×10^6 cfu/ml; the diameters of filter paper were 5 mm, and for the experiments, flat plates were incubated at 37 °C (bacterium) for 16–18 h. Their inhibition diameter (including filter paper) was measured with a vernier caliper [11].

2.7.2. Micro-dilution antibacterial assay

The serial dilution technique, using 10-well micro-plates to determine the minimum inhibitory concentration (MIC) of the complex for antibacterial activity was used. Two milliliter cultures of four bacterial strains of *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* and *Bacillus subtilis* were prepared and placed in a water bath overnight at 37 °C. The overnight cultures were diluted with sterile Muller–Hinton broth. The complexes were resuspended to a concentration of 40 μg/disc (in chloroform) with sterile distilled water in a 10-well micro-plate. A similar twofold serial dilution of gentamycin (Sigma) was used as positive control against each bacterium. One hundred microliters of each bacterial culture was added to each well. The plates were covered and incubated overnight at 37 °C. Bacterial growth in the wells was indicated by a red colour, whereas clear wells indicated inhibition [11].

3. Results and discussion

3.1. Analysis of X-ray diffraction (XRD)

Figure 1 show the XRD patterns of typical samples of compound prepared by the sonochemical process. Acceptable matches are observed for both compounds indicating the presence of only one crystalline phase in the samples prepared using the sonochemical process. The average crystallite size of the as-prepared dithiocarbamate complex nanoparticles is about 50 nm, according to the Debye-scherrer formula: $D = 0.9\lambda/ \beta \cos \theta$ [12].

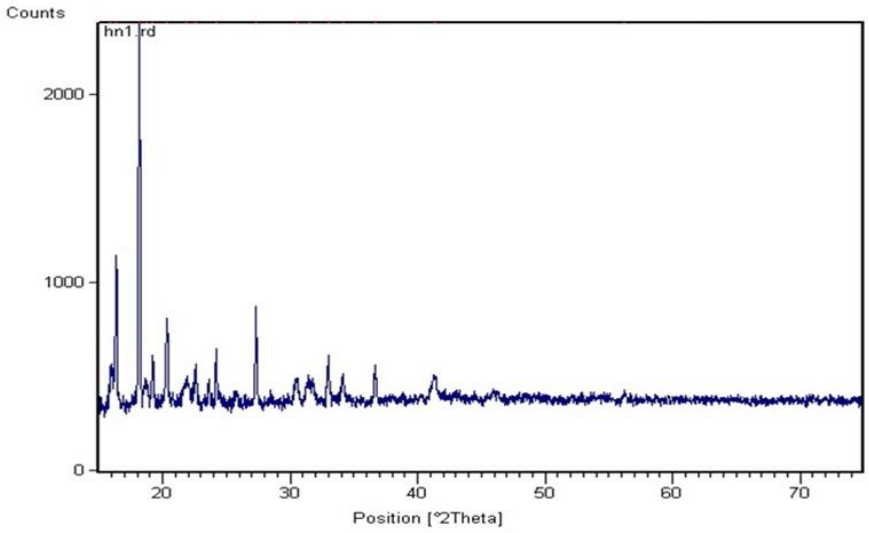

Fig.1. The XRD pattern of complex dithiocarbamate nanoparticles.

3.2. Analysis of scanning electron microscope (SEM)

Figure 2 shows the SEM image of the product, indicating that the complex nano particles are well-arranged spheres, having a mean size of 50 nm.

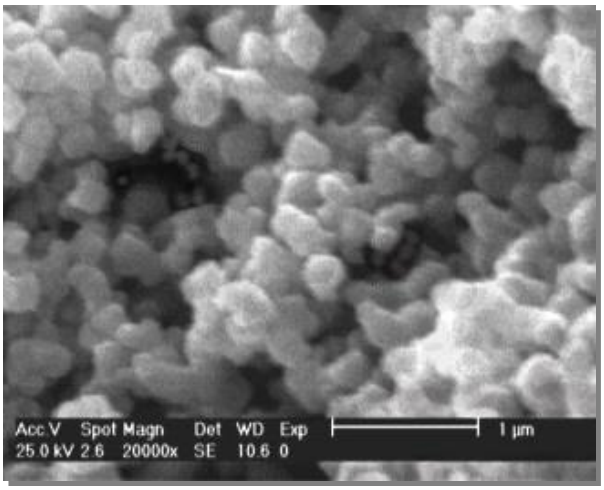

Fig.2. SEM photographs of the sample dithiocarbamate (the scale bar is 2 µm).

3.3. Analysis of infrared spectroscope (IR)

In the IR spectra of complex dithiocarbamate, the υ(CH) frequencies are observed in the 2936.69cm^{-1} and 2852.05cm^{-1} regions, whilst its deformation frequencies υ(CH) are observed at 1440.8cm $^{-1}$. The υ(C-N) stretching frequency for the thioureide group is observed at 1470.36 cm^{-1} in the ligand and in the complexes, in general at somewhat higher frequencies. Other υ (CN) vibrations, 1359.88 and 1237.38 cm^{-1}, are somewhat lower in the complexes. The υ (C-S) stretching frequency appears at 976.44 cm^{-1} in the ligand. The (S-N) vibration is observed at 855.03 cm $^{-1}$ in

the ligand as well as its complexes. The (Co-N) and (Co-S) frequencies are observed in the 400.07 cm^{-1} and 350.36 cm^{-1}region.

3.4. In vitro antifungal study

In the current study (Table 1) of some synthesized complexes were tested against pathogenic fungal strains such as *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. Ketoconazole was used as reference drug for fungi. The minimum inhibitory concentrations (MICs) by microbroth dilution assays (MDA) are 80-230 µg/mL. The complexes had highest in vitro antifungal activity against pathogenic fungal strains. The reason for the highest activity might be related to the presence of dithiocarbamate group in the [Co (pipdtc) $_2$ (1,10-phen)] complex.

Table 1. In vitro antifungal studies of the complexes

No	Complexes	Candida albicans MDA(µg/ml)	Aspergillus flavus MDA(µg/ml)	Aspergillus niger MDA(µg/ml)
1	$[Co(pipdtc)_2(1,10\text{-}phen)]$	80	95	140
2	Nano $[Co(pipdtc)_2(1,10\text{-}phen)]$	130	179	230

Where, MDA: Micro-dilution activity

3.5. In vitro antibacterial study

In the antibacterial study (Table 2) of some synthesized complexes were tested against pathogenic bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and *Bacillus subtilis* using the disc diffusion method. Gentamycin was used as reference drug for bacteria. In general, the compounds showed significant antibacterial activity and the bacterial strains with the zone of inhibition, 23 mm at minimum inhibitory concentration (MIC) of 30.0 µg/disc.

Table 2. In vitro antibacterial studies of the complexes

No	Complexes	Zone of inhibitation (mm)			
		Escherichia coli	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Bacillus subtilis***
1	$[Co(pipdtc)_2(1,10\text{-}phen)]$	13	13	11	10
2	Nano $[Co(pipdtc)_2(1,10\text{-}phen)]$	23	23	19	19

4. Conclusion

From the previous chemical analyses, the following geometrical structures were suggested (Figure 3). X-ray crystal structure [Co (pipdtc)$_2$ (1,10-phen)] and details of the crystal data, data collection and refinement parameters are indicated references [13].

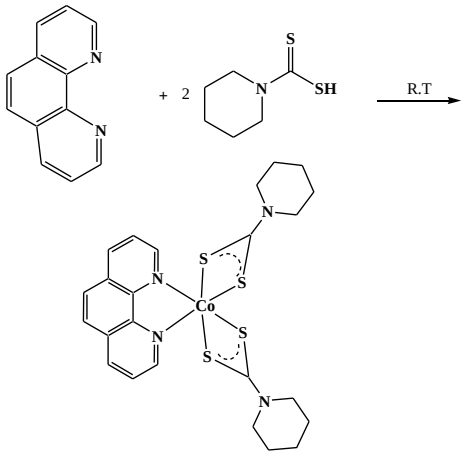

Fig.3. The structure of complex

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