SYNTHESIS, STRUCTURAL AND ANTIBACTERIAL STUDY OF NEW SILVER COMPLEX WITH 3-ACETYL-*2H* CHROMENE-2-ONE

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ABSTRACT. A new silver complex $[Ag(C_{11}H_8O_3)_2]NO_3$ was synthesized by the reaction of silver nitrate and coumarin based ligand (3-acetyl-*2H*-chromene-2-one) through solution method. The product was characterized using different analytical techniques like melting point, Infrared spectroscopy, Raman spectroscopy, powder X-ray diffraction, thermogravimetric analysis, scanning electron microscopy, atomic absorption spectroscopy and mass spectrometry. An antibacterial study of the complex was also studied for its possible use in medical treatment.

KEY WORDS: Silver complex, Acetyl coumarin, Vibrational analysis, Antibacterial study

INTRODUCTION

Coumarin and its derivatives have been isolated from woodruff vanilla grass, sweet grass, sweet woodruff, and deer's tongue. Further coumarin and its annealated derivatives are reported to possess significant antibacterial [1], coronary dilatory [2] and hypothermia activities [3]. Therefore, it became of interest to synthesize new derivatives of these systems of expected biological activities. Similarly different metal complexes of coumarin and their derivatives also exhibited biological activity against various pathogens.

Biological activity of a complex is not based only organic ligand but it is also affected by the oxidation state of the metal. These properties provide a versatile platform for drug design that is now being exploited in several areas. For centuries, silver compounds have been known to possess interesting biological properties like antibacterial properties [4-7]. Also, they were popular remedies for tetanus and rheumatism in the 19th century and for colds and gonorrhea before the advent of antibiotics in the early part of the 20th century [8]. Additionally, silver compounds used for treating mental illness, epilepsy, and nicotine addiction [9, 10]. Furthermore, they have re-emerged as a viable treatment option for infections encountered in burns, open wounds, and chronic ulcers [11-14].

In this study, acetyl coumarin was synthesized and studied against various gram +ve and gram –ve bacteria. In order to study the affect of silver metal on above mentioned organic ligand and its activity is studied against different bacterial strains.

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EXPERIMENTAL

Synthesis of silver complex

A solution of 1 mmol (0.188 g) 3-acetyl-2H-chromene-2-one and 1 mmol (0.04 g) sodium hydroxide in 10 mL water was added to a solution of 1 mmol (0.108 g) silver(I) nitrate in 10 mL water and stirred over a period of 15 min at room temperature. The coloured precipitates are formed which were then filtered off, washed with cold water and ethanol and dried in vacuum over calcium chloride at room temperature.

Melting point was determined using Scientific Electrothermal melting point apparatus (1401Q). The vibrational studies of the complex were performed on Nicolet 6700P Infrared Spectrometer (USA). The structural parameters were calculated using PANalytical (X'Pert PRO) diffractometer (using Cu-Kα radiation). Thermal measurements were performed on TGA/DSC (Q600) purging nitrogen gas using alumina crucible. Mass spectrum of complex was obtained on Mass spectrometer (1200 series, Agilent technology, with scanning speed up to 5250 μs, quadrupole, mass range m/z = 2-3000) while AAS experiments were performed on Atomic Absorption Spectrometer (Varian AA240). Metal-ligand vibrational bands were studied on Raman spectrometers (Renishaw 1000). SEM micrographs were obtained on S3700N (HITACHI), Japan.

RESULTS AND DISCUSSION

Melting point study

The melting point of the complex was performed using melting point apparatus. The results showed that melting point of silver complex was not sharp but in a range of 345-355 °C which is also not comparable with that of reactants, i.e. $AgNO_3$ (212 °C) and 3-acetyl-*2H*-chromene-2-one (120 ºC) which help to conclude that reaction was completed and a new phase was obtained.

Infrared studies

The presence of ligand was verified by IR study of the synthesized complex as shown in Figure 1. The stretching vibration of C-H corresponding to alkene is observed at 3030 cm^{-1} [15]. While the stretching bands observed at 2935 cm^{-1} and 2980 cm^{-1} are due to C-H (-CH$_3$ group) [16] which were further verified by symmetrical deformation at 1415 cm^{-1} and 1552 cm^{-1} in both IR and Raman anaylsis. An overtone band of 974 cm^{-1} appeared at 1937 cm^{-1}. The vibration at 1726 cm^{-1} is assigned to stretching bands of C=O [17, 18] which is further confirmed by Raman measurement at 1675 cm^{-1} and 1745 cm[-1][19]. The stretching vibration of C=C (benzene ring) appeared at 1609 cm^{-1}. The bending vibration of C-H (alkane) is shown at 1452 cm^{-1} and that for alkene with out of plane is also shown in the range of 1000 cm^{-1} to 650 cm^{-1}. The vibrations appeared in the range of 1300 cm^{-1} to 1000 cm^{-1} corresponds to C-O-C [20, 21]. It is reported that vibrational bands of Ag-O are lying in the range of 253-205cm^{-1} [22, 23] which is out of limits of IR range. The IR spectrum shows that there is no band assigned to –OH which clue the absence of water molecules in the synthesized product. The bands observed in the range of IR region (1609-637 cm^{-1}) which are correlated to C-C stretching vibration and comparable with literature [24]. In this study, the vibrations observed at in the region of 1620-600 cm^{-1} in both IR and Raman measurements are due to the ring vibrations.

Raman analysis

The most significant peaks of silver lattice, i.e. at 95 cm^{-1} and 146 cm^{-1} cannot be observed in Raman pattern [25] which indicates the absence of elemental silver in the product. The peaks observed in the range of 900-1100 cm^{-1} are attributed to the Ag-O stretching frequencies [26].

The raman vibration at 950 cm^{-1} are commonly related to chemisorbed oxygen species [27-29] as shown in Figure 2.
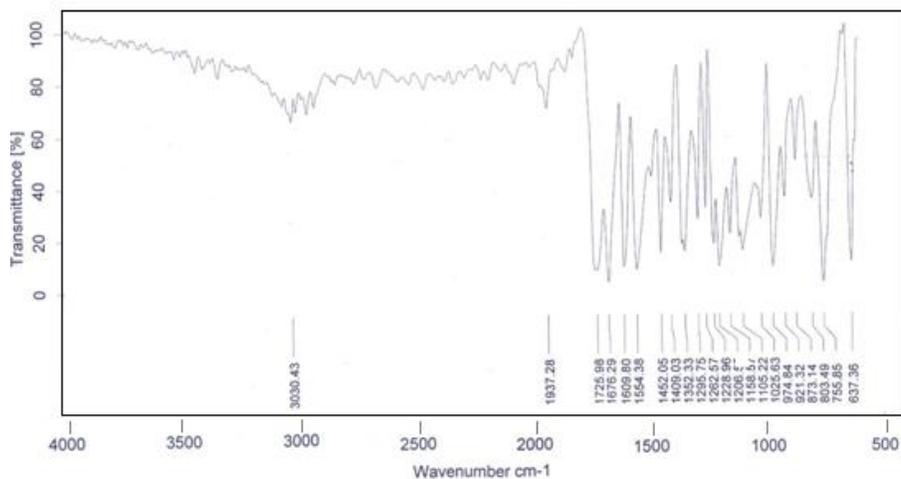
Figure 1. IR spectra of silver complex with 3-acetyl-*2H*-chromene-2-one
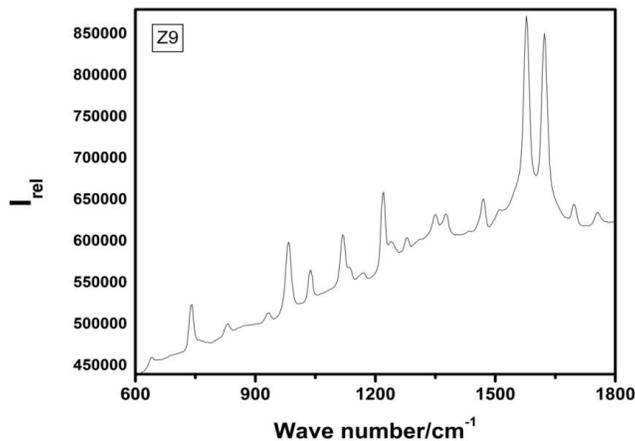
Figure 2. Raman spectrum of silver complex.

Powder X-ray diffraction analysis

The X-ray powder diffraction pattern of silver complex with 3-acetyl-*2H*-chromene-2-one was obtained on X-ray powder diffractometer under 45 kV/40 mA X-ray, $2\theta/^0$ scanning mode, fixed monocharomator and with a range from $2\theta/^0 = 10$ to 90 with a step of 0.03 degree for a period of 30 min is shown in Figure 3. Powder X-ray diffraction results of synthesized product was obtained on powder diffractometer for half an hour with a step of 0.03º along fixed monochromator for 2θ value (10º-90º). A total eight peaks were targeted and calculated their miller indices shown in Table 1.
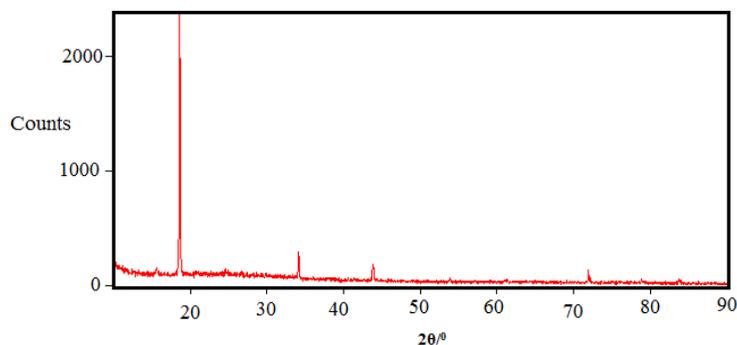
Figure 3. XRD pattern of silver complex with 3-acetyl-*2H*-chromene-2-one.

Table 1. Calculation of miller indices through XRD diffraction pattern.

$2\theta/^0$	$\theta/^0$	$Sin^2\theta$	$1xSin^2\theta/Sin^2\theta_{min}$	$2xSin^2\theta/Sin^2\theta_{min}$	$3xSin^2\theta/Sin^2\theta_{min}$	Whole integers	*hkl*
15.6758	7.8379	0.01859	1	2	3	3	111
18.6587	9.3293	0.02627	1.4131	2.8262	4.2393	4	211
34.0831	17.0415	0.08588	4.6196	9.2392	13.8588	14	321
42.7785	21.3892	0.13300	7.1543	14.3086	21.4629	21	421
53.8968	26.9484	0.20537	11.0473	22.0946	33.1419	33	522
71.1132	35.5566	0.33815	18.1898	36.3796	54.5694	54	721
79.7329	39.8664	0.41088	22.1022	44.2044	66.3066	66	811
85.5229	42.7614	0.46096	24.7961	49.5922	74.3883	74	831

To observe the novelty of synthesized complex, a comparison was made between the calculated pattern and reported patterns using peak search method. The measurement showed that peaks present at 15.6758, 18.6587, 34.0831, 42.7785, 53.8968, 71.1132, 79.7329, 85.5229 ($2\theta/^o$) exhibit miller indices as 111, 211, 321, 421, 522, 721, 811, 831 respectively that confirm the uniqueness of the pattern. The material parameters like particle size, dislocation line density and strain of the synthesized product were also calculated as shown in Table 2.

Table 2. Calculation of material parameters of Ag complex.

$2\theta/^0$	FWHM/$2\theta°$	Intensity/counts	d-spacing/Å	Grain size (D)/nm	Dislocation Density (δ)/ lines/cm^{-2}	Strain (S)/ lines^{-2}cm^{-4}
15.6758	0.4723	1.75	5.65324	2.9639	0.11383	0.1169
18.6587	0.1181	100.00	4.75565	11.9016	0.00705	0.0291
34.0831	0.1476	10.14	2.63060	9.8266	0.01035	0.0352
42.7785	0.1771	5.73	2.06789	9.0328	0.01225	0.0383
53.8968	0.2362	1.08	1.70114	1.7449	0.32844	0.1986
71.1132	0.1771	3.17	1.31344	11.8305	0.00714	0.0293
79.7329	0.3542	0.60	1.21288	5.1013	0.03842	0.0679
85.5229	0.4320	0.78	1.15351	6.4310	0.02417	0.0539

Table 2 showed that the grain size was found to be in the range of 11.9016 nm at $2\theta/^0$ = 18.65 to 2.9639 nm at $2\theta/^0$ = 15.67. Dislocation density values are smaller which indicate the purity of a substance. Variations in different structural parameters are shown in the Figure 4.
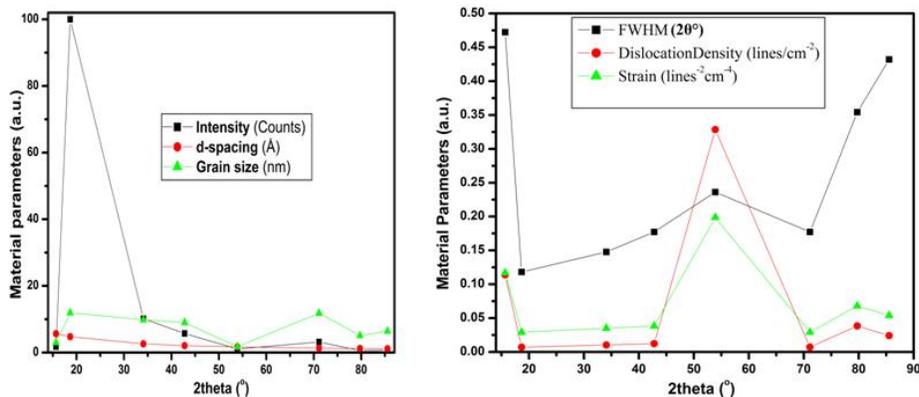
Figure 4. Graphical relations between 2θ° and material parameters.

Thermal gravimetric analysis

The sample was run on thermogravimetric analyzer (TGA/DSC-Q600) and obtained decomposition pattern as shown in Figure 5.
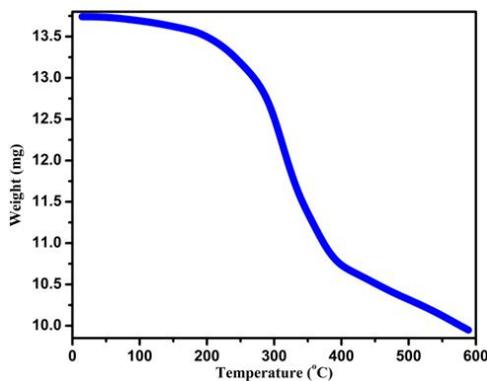
Figure 5. TGA curve of Ag metal with 3-acetyl-*2H*-chromene-2-one

Thermal analysis showed a first weight loss with fast decomposition from 200 ^{0}C to 380 ^{0}C followed by second weight loss in the range of 400 ^{0}C to 600 ^{0}C. In 1st weight loss, one of the two ligands attached to the silver metal atom may be removed and in the 2nd weight loss, second or residual ligand is expected to remove. The remaining residue was undetermined.

Electrospray ionization-mass spectrum (ESI-MS)

Mass spectrum was recorded on ESI-MS spectrometer to ensure the proposed structure of the product as shown in Figure 6.
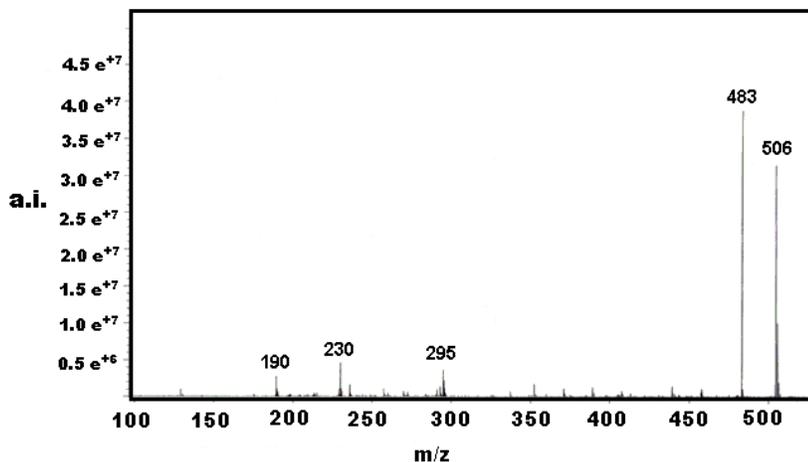
Figure 6. Electrospray ionization-mass spectrum (ESI-MS) of silver complex.

A molecular ion peak was observed at m/z 483.4 which was co-related to ionic specie $[C_{20}H_{14}AgO_6 + H]^+$. Some extra peaks were also found due to impurities or due to by-product formed during decomposition. A sharp peak of the sodium salt of the complex $[C_{20}H_{14}AgO_6 + Na]^+$ was found at m/z 506.0. Thus proposed structure of product was confirmed from molecular ion peak $[M+1]^+$ in the spectra.

Atomic absorption measurement

Atomic absorption measurement was performed to find the metal to ligand ratio (by weight %) in the synthesized product. The sample solutions and standard solutions were prepared as shown in as shown in Table 3.

Table 3. Concentration of silver metal complexes obtained experimentally.

S. No.	Sample description	Amount of metal estimated by AAS (ppm)
1	Sample 1	27.2
2	Sample 2	18.2
3	Sample 3	24.2

To determine an exact formula, experimental result was compared with theoretical one. An atomic absorption analysis shows that metal/ligand (wight %) in the synthesized complex is 24.2% which is comparable with the theoretical one (22.3%) of complex as shown in Table 4.

Table 4. Expected metal to ligand ratio.

S. No.	Ag metal	Ligand	Total weight	Metal (%)
1	1	1	296	36.4
2	1	2	483	22.3
3	1	3	671	16.1
4	1	4	860	12.5
5	1	5	1048	10.3

The result shows that metal/ligand is 1:2 hence the proposed formula of the complex is as $Ag(C_{11}H_8O_3)_2$.

Scanning electron microscopic (SEM) studies

SEM micrographs were obtained in the range of 50 µm to 5 µm using Variable Pressure Scanning Electron Microscope (TESCAN Vega LMU) as shown in Figure.7.
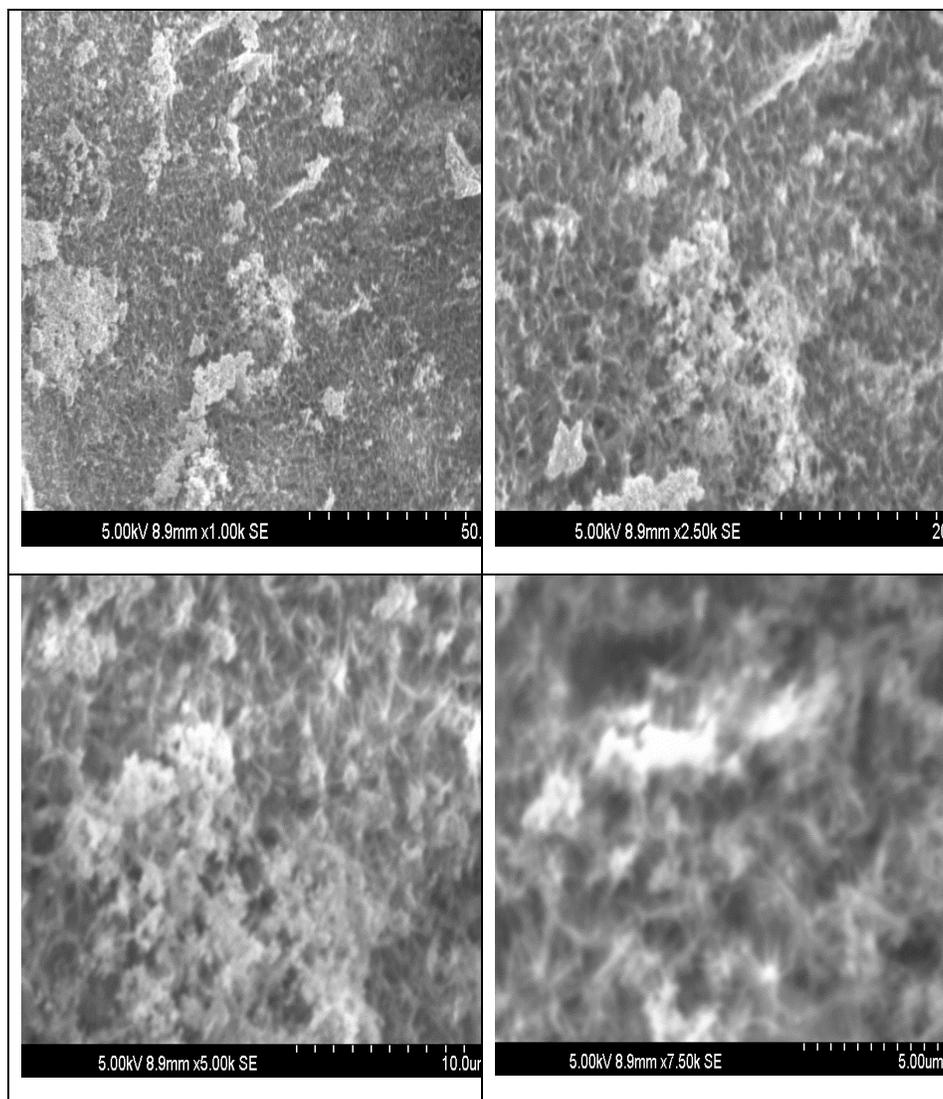
Figure 7. SEM micrographs of silver complex (with resolution 5 µm to 50 µm).

The results show that the particles of the synthesized complex exist in form of cluster having smooth surface. Secondary electron images of new silver complex show that surface morphology of product is in the range of range of micron. Spatial distribution of the particles in the material shows that they are homogenously, equally and well dispersed throughout the bulk material. The more bright regions might be due to direct interaction of light with particles.

Antibacterial activity of silver complexes

Different gram positive and gram negative bacterial strain experiments were performed using ligand and silver complex as shown in Table 5.

Table 5. Antibacterial study of silver complex.

Name of compound	Diameter of inhibition zone (mm)					
	Gram positive			Gram negative		
	S. aureus	*B. subtilis*	MRSA	*E. coli*	*P. auruginosa*	*S. typhi*
Ligand	28	18	-	17	-	-
Ag-complex	28	11	15	12	-	-

Note: Mean inhibition zones are measured in mm.

The complex was very effective against *S. aureus*, MRSA, *E. Coli* and *B. Subtilis* and reluctant their growth to a greater extent but ligand did not diffident the growth of MRSA. Both ligand and product could not inhibit the growth of *P. auruginosa* and *S. typhi*. It is cleared from above discussion that an activity of the ligand (3-acetylcoumarin) has been enhanced with an addition of Ag-metal atom.

The MIC results of silver complex against these bacteria are shown in Table 6. An experimental value using ligand is 20 µg MIC value for *S. aureus*, 15 µg for *B. subtilis* and 10 µg for *E. coli*. The lower MIC values for silver complex gave clue that activity of the product is increase by silver against pathogens.

Table 6. MIC results of silver metal complexes against these bacteria

Name of compound	Diameter of inhibition zone (mm)					
	Gram positive			Gram negative		
	S. aureus	*Bacillus subtilis*	MRSA	*E. coli*	*P. auruginosa*	*S. typhi*
Ligand	20 µg	15 µg	-	10 µg	-	-
Ag-complex	20 µg	10 µg	10 µg	5 µg	-	-

CONCLUSION

A new complex $[Ag(C_{11}H_8O_3)_2]NO_3$ is obtained. The structural studies of the complex were done using X-ray measurement. The material parameters of complex like crystallite size, dislocation line density, stress have been calculated. Results show that activity of ligand has been increased after the complex formation.

ACKNOWLEDGEMENT

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