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Synthesis, Characterization and Antimicrobial Activity Studies of Chlorocobaloximes with Neutral Bases Containing Amine Functionality

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The inorganic cobaloximes of type [Co(Cl)(dmgH)$_2$B]; where dmgH = dimethyl glyoxime and B = neutral bases: glycine, ethyl amine, 2-aminopyridine, 4-aminopyridine, 2,6-diaminopyridine, aniline and 1-napthylamine have been synthesized. The synthesized cobaloximes were characterized by IR, UV-visible, ^{1}H NMR and ^{13}C NMR spectroscopic techniques. The cobaloxime complexes were screened for their antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* by the zone of inhibition test, biofilm eradication on biomaterial using catheter and modified Congo red agar method. It has been found that the cobaloxime complexes exhibit inhibition against both gram positive and gram negative bacteria and the cobaloximes showed better inhibition towards Gram-negative bacteria compared to Gram-positive bacteria.

Keywords: Chlorocobaloximes, Dimethyl glyoxime, Amine, Aminopyridine, Antibacterial studies, Biofilm.

INTRODUCTION

In recent years, a lot of importance has been devoted to the chemistry of cobaloximes, since they have been considered as the better model complexes of coenzyme vitamin B_{12} [1-5]. Cobaloximes find their applications in various fields and they have been used as electrocatalaysts and photocatalysts for hydrogen evolution reaction [6-13]. Their application in organic transformation and polymer chemistry is well established [14-24]. It has been observed that small changes in the equatorial and axial ligands alter the properties of the cobaloximes [25-27] and so there is a continuous interest in the synthesis of cobaloximes with new structural features [28-30].

Recently, Milione *et al*. [31] investigated the influence of axial ligand in a series of cobaloximes and found that the bond between cobalt atom and the axial ligand is predominantly ionic in character. Lemire *et al*. [32] reported that the introduction of the metal ions increases the antibacterial activity of drugs. It has also been shown that the metal complexes show better activity compared to the free ligand [33,34]. The antibacterial activity of vitamin B_{12}-peptide nucleic acid conjugates showed that the conjugates are stable in biological media [35]. *Staphylococcus aureus* remains a precarious pathogen in humans [36], moreover, Zheng *et al*. [37] used the alkoxy derivatives of cobalt complexes as an antibacterial agent for methicillin-resistant *Staphylococcus aureus* (MRSA). Now-a-days, there is a large increase in resistance by bacteria against the antibiotics and MRSA is usually resistant to multiple antibiotics and these infections are difficult to treat [38]. Similarly, multi-antibiotic resistant *Acinetobacter baumannii*, is now considered to be spread from the hospitals [39,40].

Canpolat *et al*. [41] showed that the antibacterial activity of chloro(aquo)cobaloxime complexes were effective against *Enterobacter aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, but were less effective than azithromycin. Dayalan *et al*. [42,43] studied the antimicrobial activity of halo cobaloximes using well method. Biofilms play a pathogenic role during infections and are resistant to conventional antimicrobial treatment [44], which necessitates the importance of developing new antibiotics. In recent times, cobalt complexes have been utilized for the eradication of biofilms and it has not been explored [45-47]. So more studies are required to find the antibacterial activity of cobaloximes and the present study deals with the synthesis of chlorocobaloximes [Co(Cl)-

$(dmgH)_2B$] with three different types of bases (dmgH = dimethyl glyoxime and B = neutral bases: glycine, ethyl amine, 2-aminopyridine, 4-aminopyridine, 2,6-diaminopyridine, aniline and 1-napthylamine) and studying their antimicrobial activity using zone of inhibition method and eradication of biofilm using catheter and Congo red test.

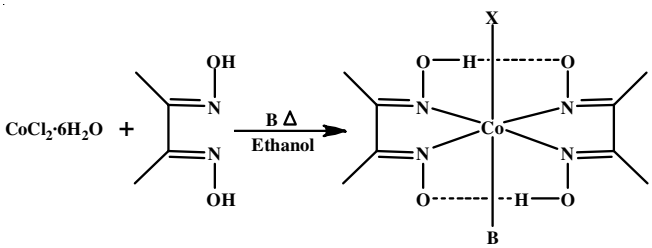
EXPERIMENTAL

Cobalt chloride hexahydrate, dimethyl glyoxime, glycine, ethyl amine, 4-aminopyridine, 2-aminopyridine, 2,6- diaminopyridine, aniline and all other chemicals were purchased from Sigma-Aldrich or Merck and used without any further purification. All solvents were purchased commercially with reagent grade quality. TLC graded silica gel was used to prepare chromatographic plates for monitoring the reaction. The visualization of the spots was done using iodine vapour. IR spectroscopic studies were done by Shimadzu Prestige 20 IR spectrometer using KBr pellets. UV-Visible spectra were recorded on Jasco V-630 spectrophotometer using quartz cells in methanol and DMSO of 1×10^{-4} M. The NMR spectra were recorded using JOEL 400MHz spectrophotometer (^{1}H: 400.13 MHz, ^{13}C: 100.62 MHz) using NMR tubes with 0.5 mm OD.

Antimicrobial activities of the complexes were determined using different microorganisms by anti-biofilm, catheter and Congo red methods. The microbial strains such as *S. aureus* and *A. baumannii* were obtained from the Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore, India.

Synthesis of chlorocobaloximes ($ClCoL_2B$)

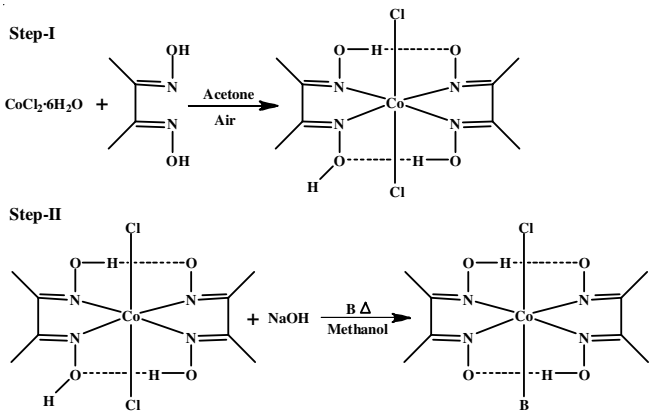
Method-1: To a boiling ethanol solution (150 mL), $CoCl_2 \cdot 6H_2O$ (5 g, 21 mmol) and dimethyl glyoxime (5.5 g, 47 mmol) were mixed, refluxed for 2 h and then the solution was cooled to room temperature. Neutral base (45 mmol) was added, again refluxed for 2 h (**Scheme-I**) and then the solution was cooled to room temperature. Air was passed through the solution for 2 h, a brown precipitate was filtered through Büchner funnel, washed with ethanol and dried in open atmosphere.



Scheme-I: Synthesis of chlorocobaloximes by method-1

Method-2 (step-1): Dimethyl glyoxime (1.10 g, 9.5 mmol) was added into the stirred solution of cobalt chloride (1.50 g, 4.59 mmol) in acetone (25 mL). Gentle stream of air was passed through the solution and the product was obtained as green solid. After 30 min, the solution was cooled in ice bath, filtered and washed with 2 × 10 mL of cold acetone (Yield: 1.4 g, 61.9%)

Step-2: A green microcrystalline product [$Co(Cl)_2(dmgH)(dmgH_2)$] (1 g, 2.7 mmol) from the first step was suspended in methanol (20 mL) followed by the addition of base (5.5 mmol) into the suspension. The mixture was stirred well until the green solid disappears and replaced by brown crystalline solid. Water was added with stirring and then the suspension was cooled in ice bath for 10 min. The product was collected by suction filtration and washed with water/methanol and diethyl ether (**Scheme-II**). FT-IR (KBr pellets ν_{max}/cm^{-1}): complex (**1**): 1239 δ(N-O), 1567 δ(C=N), 506 δ(Co-N), 3251 δ(O-H--O), 1394 $\delta_s(CH_3)$, 1497 $\delta_{as}(CH_3)$. Complex (**2**): 1241 δ(N-O), 1568 δ(C=N), 510 δ(Co-N), 3204 δ(O-H--O),1366 $\delta_s(CH_3)$. Complex (**3**): 1244 δ(N-O), 1560 δ(C=N), 520 δ(Co-N), 3418 δ(O-H--O), 1361 $\delta_s(CH_3)$, 1485 $\delta_{as}(CH_3)$. Complex (**4**): 1236 δ(N-O), 1534 δ(C=N), 515 δ(Co-N), 3372 δ(O-H--O),1374 $\delta s(CH_3)$. Complex (**5**): 1239 δ(N-O), 1568 δ(C=N), 510 δ(Co-N), 3327 δ(O-H--O), 1360 $\delta_s(CH_3)$, 1450 $\delta_{as}(CH_3)$. Complex (**6**): 1240 δ(N-O), 1566 δ(C=N), 509 δ(Co-N), 3258 δ(O-H--O), 1367 $\delta_s(CH_3)$, 1439 $\delta_{as}(CH_3)$. Complex (**7**): 1240 δ(N-O), 1565 δ (C=N), 515 δ(Co-N), 3553 δ(O-H--O), 1372 $\delta_s(CH_3)$, 1440 $\delta_{as}(CH_3)$.



Scheme-II: Synthesis of chlorocobaloxime [B = neutral bases: glycine (**1**), ethylamine (**2**), 2-aminopyridine (**3**), 4-aminopyridine (**4**), 2,6-diaminopyridine (**5**), aniline (**6**) and 1-napthylamine (**7**)]

UV-Vis (λ_{max}, nm in (C_2H_5OH and DMSO, π-π*, n-π*) (**1**): 208, 220, 251; (**2**): 228, 248; (**3**): 263, 295; (**4**): 206; (**5**): 206, 240, 304; (**6**): 208, 223; (**7**): 212.

^{1}H NMR (DMSO-d_6, TMS, 400 MHz, δ ppm) (**1**): 2.70 (4H, s, $\delta_s(CH_3)$), 3.35 (2H, s, $\delta_{as}(CH_3)$); (**2**): 2.70 (4H, s, $\delta_s(CH_3)$), 0.77(3H, t, 7.2 Hz, $\delta_{as}(CH_3)$), 1.60 (2H, q, 7.2 Hz, $\delta_{as}(CH_3)$); (**3**): 2.24 (4H, s, $\delta_s(CH_3)$), 6.41-6.46 (2H, m, Ar), 7.33 (1H, t, 7.6 Hz, Ar), 7.88 (1H, d, 4.0 Hz, Ar); (**4**): 2.30 (4H, s,$\delta_s(CH_3)$), 6.35 (2H, d, 5.2 Hz, Ar), 7.23 (2H, d, 5.2Hz, Ar), 6.82(2H, s, $\delta_{as}(CH_3)$); (**5**): 2.27 (4H, s, $\delta_s(CH_3)$), 6.21(1H, d, 8.0 Hz, Ar), 7.02-7.16 (2H, m, Ar); (**6**): 2.20 (4H, s, $\delta_s(CH_3)$), 6.47-6.49 (2H, m, Ar), 7.09-7.12 (3H, m, Ar); (**7**): 2.02 (4H, s, $\delta_s(CH_3)$), 7.07-8.03 (7H, m, Ar).

^{13}C NMR (DMSO-d_6, TMS, 100 MHz, δ ppm): (**1**): 12.6 (-CH_3 of dmgH), 152.4 (-C=N-), 170.3 (C=O); (**2**): 12.6 (-CH_3 of dmgH), 151.7 (-C=N-), 37.1(-CH_2-), 14.9 (-CH_3); (**3**): 12.4 (-CH_3 of dmgH), 111.7, 137.3, 147.6 (Ar), 152.1 (-C=N-); (**6**): 12.3 (-CH_3 of dmgH), 127.9, 125, 122 (Ar), 151.3 (-C=N-); (**7**): 12.2 (-CH_3 of dmgH), 124.8 (Ar), 151.9 (-C=N-).

Antibacterial activity: The test microorganisms used for the antibacterial studies were methicilin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. The

following three tests were carried out to understand the antimicrobial activity of synthesized cobaloximes (**1-6**). The anti-bacterial efficiency of the cobaloximes was found by performing agar well diffusion method using Muller-Hinton Agar (MHA). Four hour bacterial cultures of *A. Baumannii* and MRSA were swabbed using sterile cotton swabs onto petri-plates containing Muller-Hinton Agar. In the swabbed plate, wells were punctured into the agar. The cobaloximes dissolved in DMSO (5 mg/mL) were added at different concentrations (100, 200, 300, 400 µg/mL) to respective wells and examined for zone of inhibition after incubation at 37 ºC for 24 h.

Biofilm eradication on biomaterial: The suction catheter segments were incubated along with the bacterial suspension and cobaloxime at concentrations of 500 µg/mL at 37 ºC for 24 h. The reduction of biofilm was analyzed by comparing the colour intensity of deposit on the biomaterial, which was treated and untreated with cobaloxime. Biomaterial incubated without cobaloxime was used as control. The experiments were performed twice to confirm the results. Biomaterials were then washed in phosphate buffer saline (PBS) to remove the adhering bacterial cells. The biomaterial was then air dried and stained with 0.1% crystal violet.

Modified Congo red agar method: Modified Congo red agar method for screening of biofilm formed by *A. Baumannii* and MRSA requires the use of a specially prepared solid medium Brain Hearth Infusion Broth (BHI) supplemented with 5% sucrose and Congo red along with NaCl (1.5%) and glucose (2%) was used. Cobaloxime complex (500 µg/mL) was added to the Congo red dye. It was then mixed along with agar media after it had cooled. Control plates containing Congo red stain without cobaloxime were also prepared. Cobaloxime complexes were then inoculated into the medium and incubated at 37 ºC for 24 h.

RESULTS AND DISCUSSION

Chlorocobaloximes {$Co(Cl)(dmgH)_2B$}, with three types of bases namely (i) aliphatic amines (ethylamine and glycine), (ii) aniline containing bases (aniline, 1-napthylamine) and (iii) amine substituted pyridine bases (2-aminopyridine, 4-amino-pyridine, 2,6-diaminopyridine) have been synthesized by two methods. Yamazaki [47] reported the synthesis of chloro-cobaloxime with pyridine as axial ligand with 63% of yield. In the first method, slightly modified procedure was adopted. The dichlorocobaloxime, complex $[Co(Cl)_2(dmgH)(dmgH_2)]$, prepared in method-2 (step-1) has been used as a precursor for the synthesis of chlorocobaloximes. The dichlorocobaloxime turns dark-brown on treatment with axial ligands (bases). Better yield was obtained in method-2. All the synthesized chloro-cobaloximes were dark-brown in colour and microcrystalline in nature. The percentage yield of synthesized cobaloximes synthesized are given in Table-1.

TABLE-1
% YIELD OF CHLOROCOBALOXIMES BY METHOD-2

Compound No.	Yield (%)
1	52.6
2	46.2
3	74.2
4	59.1
5	56.1
6	68.4
7	60.2

IR studies: The characteristic frequencies are given in Table-2. The peaks around 1091 and 1242 cm^{-1} have been assigned to N-O stretching and for the free dimethylglyoxime ligand the peak appears around 1143 cm^{-1}. Free dimethyl glyoxime does not have peak around 520-490 cm^{-1}, but after complexation the Co-N stretching appears around 513 cm^{-1}. The Co-N stretching in dichlorocobaloxime shows peak at 507 and after the addition of base the position of the peak got shifted [42]. The bands around 410 cm^{-1} confirms the Co-Cl bond formation. The bands around 1380 and 1440 cm^{-1} are due to symmetric and asymmetric vibrations corresponding to the methyl group of dimethylglyoxime, respectively.

The C=N stretching of the oxime in the complexes has been observed in the range of 1600-1550 cm^{-1}. The peak around 1568 cm^{-1} due to the C=N stretch of $dmgH_2$ shifts to the lower frequency. For the free base of 2-aminopyridine and aniline, the N-H peak exists around 3400 cm^{-1}. When the electron donating base is coordinated to cobalt, it increases the electron density at the metal center. This facilitates back-donation from Co(III) to the nitrogen atoms of equatorial ligand resulting in increase in electron densities in C=N and N-O bonds. The facilitated back-donation from the cobalt to nitrogen atoms of dimethyl glyoxime lowers the C=N stretching frequency [41]. In dimethylglyoxime, there is a strong O-H band exist around 3205 cm^{-1} and after the complexation the peak gets shifted to 3265 cm^{-1} [42]. All the complexes show a weak broad band

TABLE-2
KEY IR SPECTRAL BANDS (cm^{-1}) OF SYNTHESIZED CHLOROCOBALOXIMES

Compound	δ(O-H···O)	δ(C=N)	$\delta_{as}(CH_3)$	$\delta_s(CH_3)$	δ(N-O)	δ(Co-N)
$Cl_2Co(dmgH)(dmgH_2)$	3731.00	–	1492.00	1379.16	1219.06	507.34
1	3251.00	1567.23	1497.79	1394.59	1239.32	506.34
2	3204.87	1568.19	–	1366.62	1241.25	510.19
3	3418.00	1560.48	1485.25	1361.20	1244.00	520.20
4	3372.68	1534.44	–	1374.34	1236.42	515.98
5	3327.30	1568.20	1450.50	1360.20	1239.30	510.10
6	3258.87	1566.00	1439.92	1367.59	1240.28	509.25
7	3553.03	1565.30	1440.20	1372.40	1240.20	515.02

*δ(C=O) = 1718.65 cm^{-1}

around 3550 cm^{-1}, which corresponds to the intramolecular hydrogen bonded O-H of $dmgH_2$. Yamazaki [47] reported the inorganic cobaloximes with *p*-toluidine and pyridine as axial ligands. Comparing to the IR result of those complexes, the same kind of bands for both complexes were observed. Presence of carbonyl group in compound **3** has been confirmed by the appearance of a strong peak at 1718.65 cm^{-1}.

UV-visible studies: The UV absorption spectra of the synthesized compounds have been recorded and the values are given in Table-3. The UV spectrum of all the complexes show an absorption in the range 206-295 nm [42]. The UV spectrum of cobaloxime with 2,6-diaminopyridine as axial base showed a shoulder at 304 and 358 nm, which may be due to the ligand to metal charge transfer (LMCT). The synthesized dichlorocobaloxime showed shoulder at 247 nm, which confirms the π-π^* transition of the coordinated dimethyl glyoxime. Other compounds show the π-π^* transition due to pyridine ring and the coordinated dimethylglyoxime. The absorption bands due to *d-d* transition in the visible region has not been observed clearly as the molar absorption coefficients of the complexes are very low and are masked by the CT transitions [48].

TABLE-3
UV-VISIBLE SPECTROSCOPIC DATA
OF SYNTHESIZED COBALOXIMES

Compound	Absorbance (nm)	Molar extinctintion coefficient (M^{-1} cm^{-1})
$Cl_2Co(dmgH)(dmgH_2)$	207, 247	0.207, 0.247
1	208, 220, 251	0.280,0.220,0.251
2	228, 248	0.228,0.248
3	263, 295	0.263, 0.295
4	266	0.266
5	206, 240, 304	0.206, 0.240,0.304
6	208, 223	0.208,0.223
7	212	0.212

^{1}H NMR studies: Proton NMR spectroscopy has been used extensively for the study of cobalt(III) complexes, especially in structural assignments and examination of *cis* and *trans* influence studies and the values are given in Table-4. The sharp singlet around 2.10-2.33 ppm corresponds to the equatorial methyl protons of dimethyl glyoxime. For complex **2**, the peaks at 1.60 ppm and 0.77 ppm are due to the protons present in the CH_2 and CH_3 group with the coupling constant value 7.2 Hz. For complex **6**, the aromatic protons appear around 6.48 and 7.11 ppm as a multiplet.

^{13}C NMR studies: The ^{13}C NMR spectroscopy has been used extensively to study cobaloximes and the values are given in Table-5. Small variations in the chemical shift are noted as the axial base was varied. The ^{13}C NMR spectrum was characterized as follows: the methyl carbon in dimethyl glyoxime for all the compounds appear around 12.4-12.6 ppm. The C=N group chemical shift appears around 151 ppm. In complex **2**, the signal at 12.63 corresponds to the methyl carbon in dimethyl glyoxime. The signal at 151.76 ppm corresponds to the C=N in the dioxime ligand and the signals at 37.16 and 14.9 ppm are due to the carbons present in the ethyl group of the complex. In complex **6**, the signal at 12.33 corresponds to the methyl carbon of dimethyl glyoxime. The signal at 151 ppm is due to the C=N in the dimethylglyoxime and the signals at 127.94, 125.05 and 122 ppm are due to the carbons present in the aromatic ring. In complex **3**, the signal at 12.46 ppm corresponds to the methyl carbon in dimethylglyoxime. The signal at 153 ppm is due to the C=N present in dimethylglyoxime and the signals at 114.1, 128.49 and 129.23 ppm are due to the carbons present in the aromatic ring.

TABLE-4
^{1}H NMR DATA OF SYNTHESIZED COBALOXIME COMPLEXES

Compd. No.	$\delta(CH_3)$	Aromatic region	$\delta_{as}(CH_3)$
1	2.14 (s)		3.35 (2H, s)
2	2.33		0.77 (3H, t, 7.2 Hz) 1.60 (2H, q, 7.2 Hz)
3	2.24 (s)	6.41-6.46 (2H, m) 7.33 (1H, t, 7.6 Hz) 7.88 (1H, d, 4.0 Hz)	
4	2.30 (s)	6.35 (2H, d, 5.2 Hz) 7.23 (2H, d, 5.2 Hz)	6.82 (2H, s)
5	2.27 (s)	6.21 (1H, d, 8.0 Hz) 7.02-7.16 (2H, m)	
6	2.20 (s)	6.47-6.49 (2H, m) 7.09-7.12 (3H, m)	
7	2.02 (s)	7.07-8.03 (7H, m)	

Antibacterial activity: In this study, the antibacterial activity against MRSA and *A. baumannii* using six synthesized complexes (**1**-**6**) were evaluated. The zone of inhibition test was conducted and when the agar plates were supplemented with antibiotics. Rifampicin and gentamycin, the inhibited area was 22 mm and 20 mm, respectively. From the zone of inhibition test, it has been identified that when the agar plates were supplemented with the complexes, the microbes used were sensitive to the complexes. DMSO was used as the solvent for dissolving the complexes. As the concentration increases the zone of inhibition of the cobaloxime complex also increases (Table-6).

When the synthesized complexes were tested against MRSA, complexes **3** and **6** showed the maximum inhibition. Complex **2** showed the minimum inhibition even at their highest concentration. Similarly, the testing of complexes against *A.*

TABLE-5
^{13}C NMR DATA OF SYNTHESIZED COBALOXIME COMPLEXES

Compound No.	CH_3 of dmgH	C=N of dmgH	Aromatic carbons	Other carbons
1	12.6	152.4	–	170.3 (C=O)
2	12.6	151.7	–	37.1 (CH_2), 14.9 (CH_3)
3	12.4	152.1	111.7, 137.3, 147.6	–
6	12.3	151.3	127.9, 125, 122	–
7	12.2	151.9	124.8	–

TABLE-6
ANTIBACTERIAL ACTIVITY DATA OF CHLOROCOBALOXIMES AGAINST HUMAN PATHOGENS

Complex	Zone of inhibition							
	MRSA				*Acinetobacter baumannii*			
	Concentration of the complex				Concentration of the complex			
	100	200	300	400	100	200	300	400
1	09	11	11	16	19	22	25	27
2	07	08	09	10	10	12	13	14
3	11	17	19	21	14	20	20	22
4	9	11	12	13	9	10	11	12
5	09	10	14	16	8	9	10	11
6	11	17	19	21	14	20	20	22

baumannii was done, where complex **1** showed the maximum inhibition and the complex **5** showed a minimum inhibition at the maximum concentration. Complexes **1**, **4** and **6** also showed inhibition against both MRSA and *A. baumannii*, respectively. In general, the complexes showed better activity against Gram-negative bacteria compared to Gram-positive bacteria.

Surface colonization and biofilm eradication on biomaterial: Among the various medical biomaterials used the suction catheters are the surface most prone to colonization. The obtained visual results showed visible biofilm eradication from the surface of suction catheter tubes at 500 µg/mL (Fig. 1).

The reduction of biofilm was clearly visible, when the catheter segments were treated with cobaloximes. On treatment with cobaloxime complexes, there was a decrease in colour intensity of the deposit on the biomaterial as shown in Figs. 2 and 3. This shows that the synthesized cobaloximes are able to reduce biofilms of *A. baumannii* and MRSA on biomaterial surface.

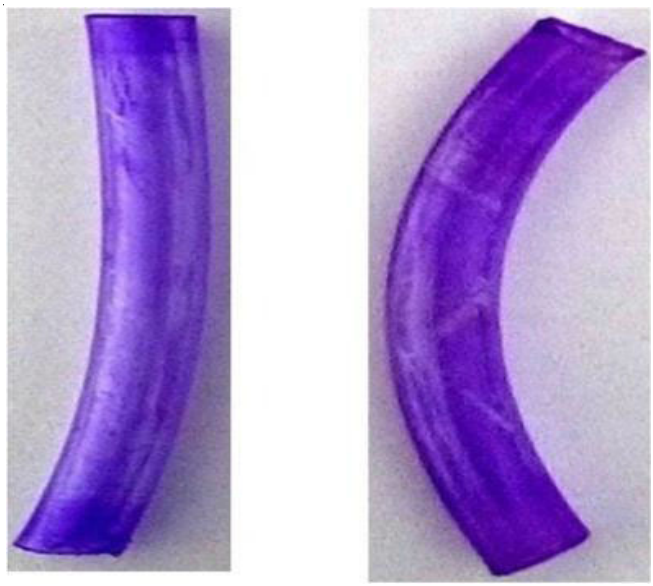
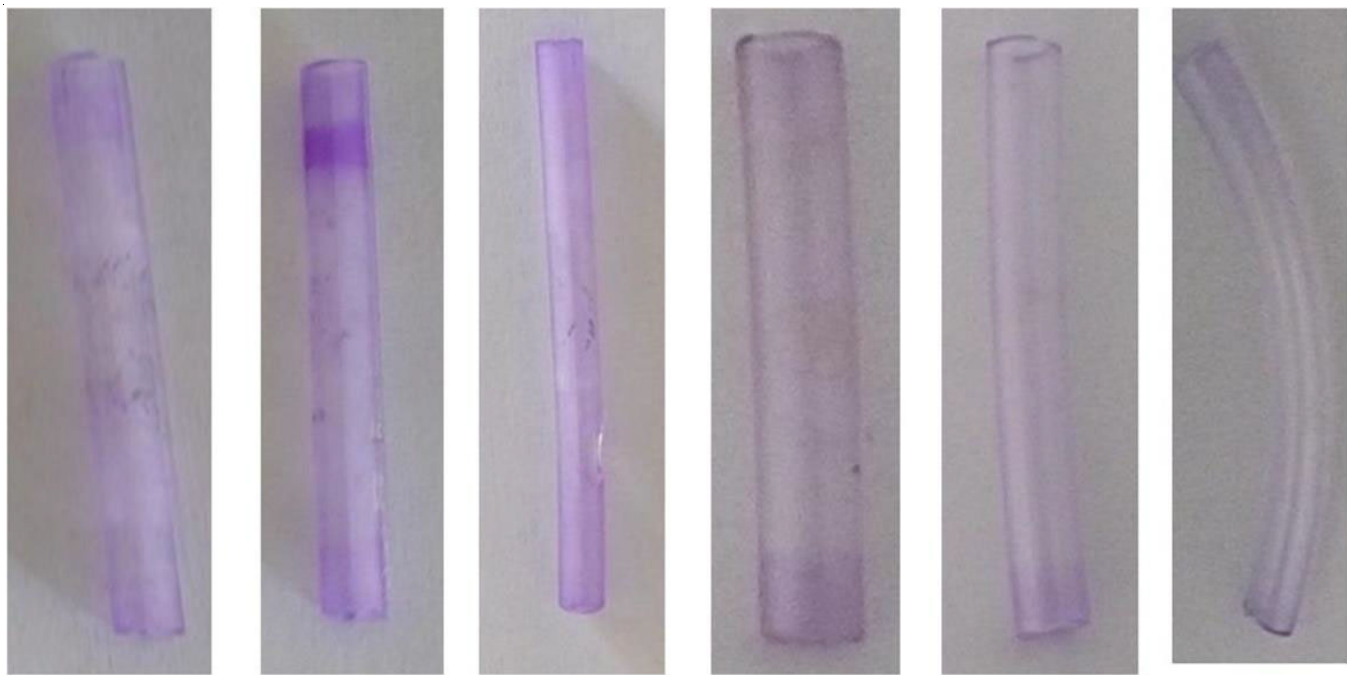


Fig. 1. Catheter segment incubated with (a) MRSA (b) with *A. baumannii*



(A) (B) (C) (D) (E) (F)

Fig. 2. Treatment of cobaloximes (A) 1 (B) 2 (C) 3 (D) 4 (E) 5 and (F) 6 against MRSA

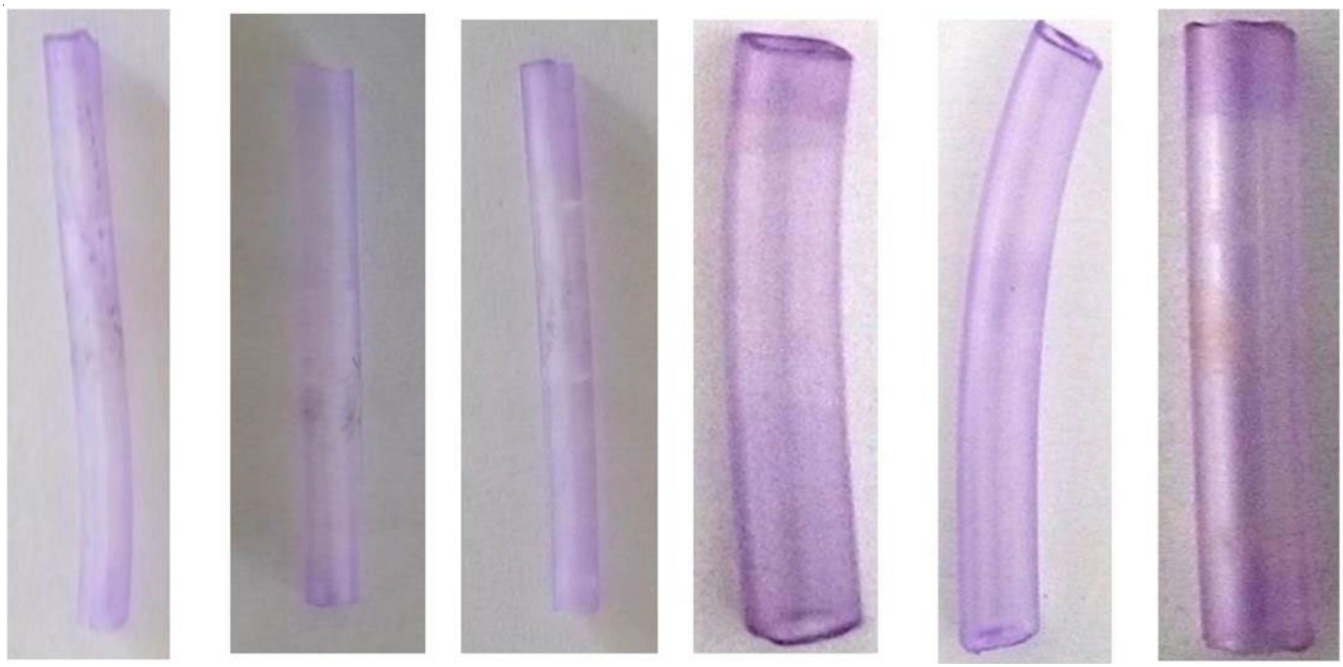


Fig. 3. Treatment of cobaloximes (A) 1 (B) 2 (C) 3 (D) 4 (E) 5 and (F) 6 against *A. baumannii*

Detection of slime production by modified Congo red agar (CRA) method: The test microorganisms (*A. baumannii* and MRSA) were screened for biofilm production by using modified Congo red agar method. When both the test microorganisms, *A. Baumannii* and MRSA were subjected to biofilm production, it was found that both the strains were strong producers of biofilm (Fig. 4). The colour change occurs towards the later stages of incubation suggesting that certain secondary metabolites released by microorganism combine with Congo red to impart black colour to the colonies indicating the slime production.

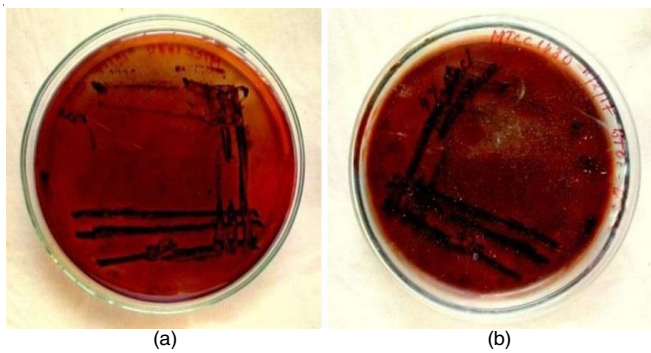


Fig. 4. Biofilm producing strains of (a) *A. Baumannii* (b) MRSA

The ability of synthesized cobaloximes to inhibit the synthesis of slime was qualitatively examined on modified CRA plate assay. The complexes at concentration 500 µg/mL was incorporated into modified CRA plates to determine whether the growing colonies could show any change in colour from black to Bordeaux red or red. The cobaloximes were able to inhibit the slime production of *A. baumannii* (Fig. 5) and MRSA (Fig. 6).

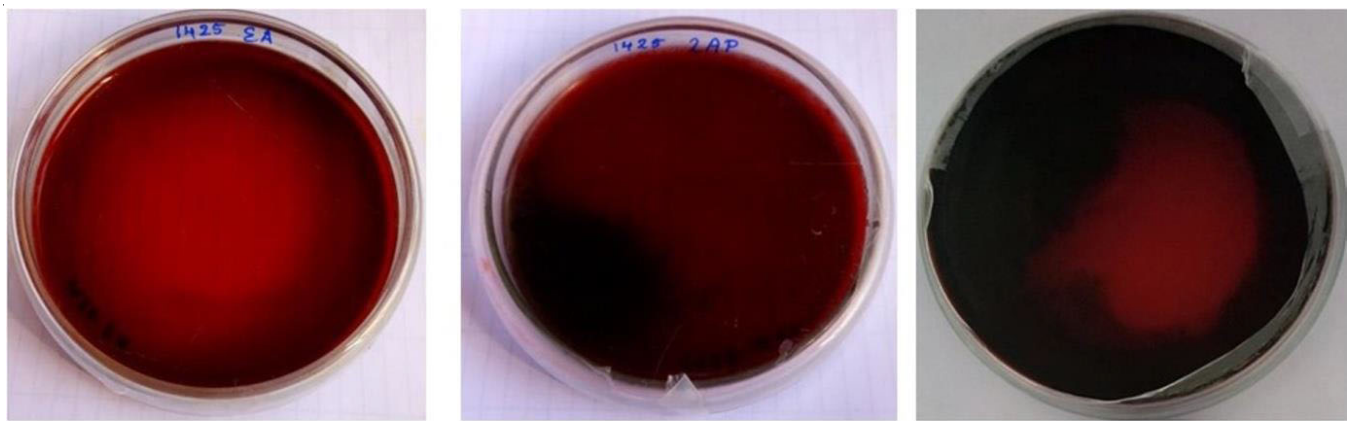
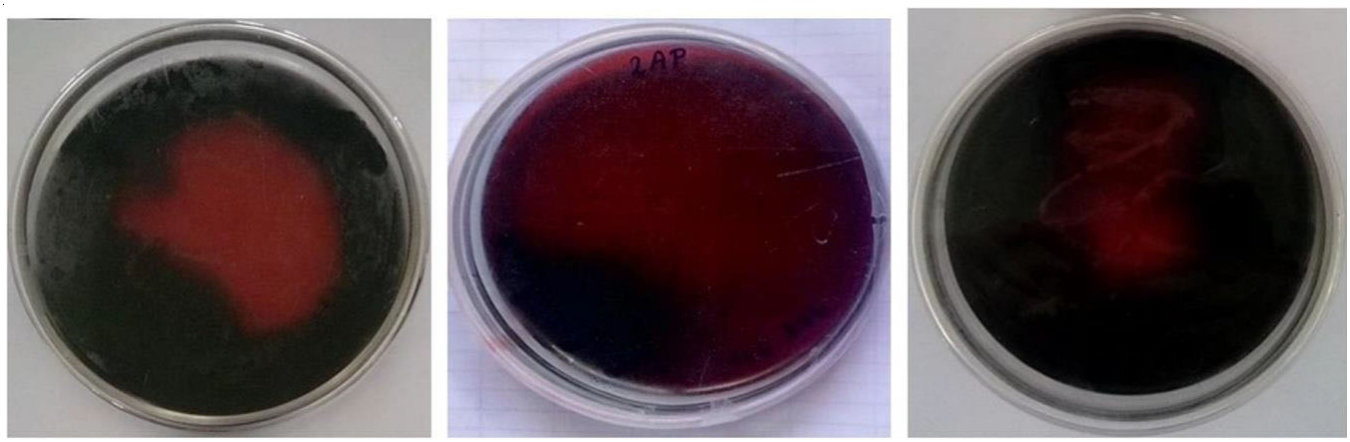


Fig. 5. Treatment of cobaloximes (500 µg/mL) (A) 2 (B) 3 (C) 6 against *A. baumannii*



(A) (B) (C)

Fig. 6. Treatment of cobaloximes (500 µg/mL) (A) 2 (B) 3 (C) 6 against MRSA

Conclusion

A series of chlorocobaloximes with various amine, pyridine and aniline substituted bases as neutral ligand have been synthesized by two methods and better yield was obtained when the complexes were synthesized by two-step process. The synthesized complexes have been characterized by IR, UV-Visible, ^{1}H and ^{13}C NMR spectroscopic techniques. The antibacterial activity of the complexes against MRSA and *A. baumannii* were studied by three different methods. The cobaloxime complexes were screened for their antibacterial activity against methicilin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* and found to exhibit inhibition against both Gram-positive and Gram-negative bacteria. The study also evaluated the efficacy of cobaloxime to inhibit the formation of biofilms on Congo red agar medium.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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