SYNTHESIS, OPTIMIZATION, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF Cu(II) AND Co(III) COMPLEXES OF BIS(2,2$^{/}$-METHYLYLIDENEPHENOL)DIAMINOETHANE

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Abstract. A new synthetic extractive protocol for the synthesis of Cu(II) and Co(III) complexes of bis(2,2$^{/}$-methylylidenephenol)diaminoethane (H$_2$BMPDE) in a single simple step was performed. The obtained data indicated the formation of complexes with 1:1 molar ratio of metal:ligand, of distorted square planar and distorted octahedral geometries. *In vitro* antibacterial screening revealed that the complexes were active against clinically important gram-negative bacteria (*Escherichia coli, pseudomonas,* and gram-positive bacterium (*Staphylococcus aureus*). The synthesis of the complexes was optimized to make it more suitable and efficient for industrial scale production.

Introduction

The metal H$_2$BMPDE type compounds have been synthesized with the attempt to discover fluorescence, antimicrobial, oxidizing and oxygen carrying activities [1-3]. The concerted spatial arrangement of the ligands around the metal ion is one of the most fascinating features of metal- coordinated systems as observed in H$_2$BMPDE –metal complexes. The review work [4] shows that five synthetic routes exist for the preparation of Schiff base metal complexes. The catalytic, biological and fluorescence properties of metal complexes are influenced by the group attached to the axial positions and other appendages as may be seen due to the presence of coordinating solvents. The designed synthetic protocols [4] could not be modelled as they all involved constituent combination method (organic synthesis). However, the extractive method, which involves the combination of several input factors at separate predetermined conditions, presents a new and simple preparatory method which can prove to be industrially important in the design of a reaction process. The response surface (RS) methodology applying Box-Behnken design is very important in modelling the interaction between two or more process variables and a number of quantitative variables as well as combination of factors at levels that will give the optimal expected yield [5].

The biological activities of H$_2$BMPDE complexes as oxygen carriers, antimicrobial agents, in dismutation of reactive oxygen species (ROS) and other biological activities have been studied by several authors [6-8]. Other studied applications of H$_2$BMPDE complexes included: as electrolyte and sensitive electrode, as red phosphorescent light emitting diode (Ph OLEDs), catalyst and electrocatalyst, magnetic material, nanoparticle and nanomaterial, oxidizing agent, corrosion inhibitor and in carbon capture [2,9]. Studies [10-14] showed that inorganic metal compounds were used as chemotherapeutic agents and that some drugs had enhanced activity when administrated as metal complexes as opposed to free organic compounds.

The aim of this study was to prepare, extract and optimize the preparation conditions using Box-Behnken design in the response surface methodology approach, characterize Cu(II) and Co(III) complexes of bis(2,2$^{/}$-methylylidenephenol)diaminoethane (H$_2$BMPDE) and the investigation of their antimicrobial bio-efficiency.

Experimental
Materials

All the chemicals and solvents used for the syntheses were of analytical grade, obtained from Merck Company and were not purified unless otherwise stated. Stock solutions of Cu(II) and Co(II) were prepared using CuSO$_4$·5H$_2$O and

$CoCl_2 \cdot 6H_2O$. Stock solutions of mineral acids (HCl, HNO_3 and H_2SO_4) were prepared by diluting the concentrated acids and were standardized using appropriate standard bases. Solutions for antibacterial studies and sensitivity tests were prepared from nutrient broth powder and Mueller-Hinton powder while aliquoted 0.5 McFarland standard was used as control [15].

Characterization methods

Design Expert software version 8.0.7.1 (Stat-ease Inc., USA, 2011) was used for optimization studies. The UV-Vis spectra of the H_2BMPDE and its metal complexes were recorded in dimethylformamide (DMF) on a Genesis 10S UV-Vis spectrophotometer. IR spectra of the ligand and the complexes were recorded on Perkin-Elmer FTIR-8400S Fourier transform infrared spectrophotometer (Shimadzu, Japan) in the range of $4000-400 cm^{-1}$ as KBr disks. Magnetic susceptibility was measured on a Johnson Matthey magnetic susceptibility balance Alfa product, Model No. MKI and diamagnetic corrections were calculated using Pascal's constant. Elemental CHN analyses were performed using Vario-Elemental Microcube ELIII. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, at room temperature, on Bruker AVANCE II 400 MHz NMR spectrometer, using tetramethylsilane (TMS) as internal reference.

Ligand synthesis

The ligand (H_2BMPDE) was synthesized by condensation of salicylaldehyde and ethylenediamine in 2:1 molar ratio (Scheme 1) [4]. In the synthesis, 22.475 g of ethylenediamine were added to 91.336 g of salicylaldehyde in a 500 mL beaker. A vigorous and spontaneous reaction occurred, producing a yellow boiling mixture. This was stirred, warmed using an electric mantle for five minutes, allowed to cool in an ice bath, and the formed crystals were collected on a 2.5 cm x 2.5 cm Whatmann filter paper and subjected twice to re-crystallization using carbon tetrachloride. The obtained golden yellow crystals were air dried. The melting point was determined to be $127 \pm 1^{\circ}C$ (literature melting point, $127-128^{\circ}C$ [2]) while the percentage yield was 64.45%.
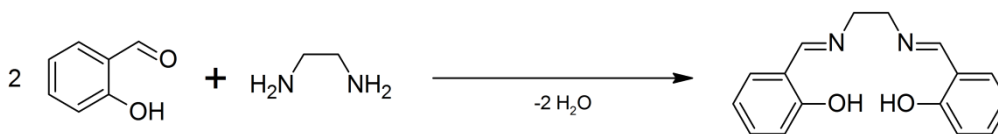
Scheme 1. Synthesis of H_2BMPDE.

Optimization studies

An aliquot of 0.1 mL of a 1000 μg/mL solution of the metal under study was pipetted into different extraction bottles. Appropriate volumes of acid (HCl, HNO_3, H_2SO_4 and $HClO_4$ respectively) were added into each bottle, as to reach a final acid concentration ranging from 0.0001 M to 2 M on final dilution with water to 5 mL. Following, 0.5 mL of 0.5% solution of H_2BMPDE and 5 mL of chloroform were added into each bottle. The phase was centrifuged at 500 rpm and separated, and its absorption was measured at 465 and 395 nm for Co(III) and Cu(II) respectively, against blank. The quantity of the metal ion coordinatively combined with H_2BMPDE, q_e (μg/mg) was calculated using Eq.(1) [16]:

$$q_e = \frac{(C_0 - C_e) \times V}{M} \qquad (1)$$

where, V– volume of metal solution (mL);
C_0– initial metal concentration (μg/mL);
C_e– equilibrium metal concentration (μg/mL);
M– mass of ligand (mg).

Experimental design

The experiments designed according to Box-Behnken central composite design (CCD) included 46 experiments of five variables at three levels (–1, 0, +1) with 3 runs at the centre point level in duplicate, recording the average value. The between values for each process variables are shown in Table 1, while the second order polynomial showing the relationship between the variables is shown in Eq.(2):

$$Z = n_o + \sum_{i=1}^{k} n_i x_i + \sum_{i=1}^{k} n_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} (n_{ij} x_i x_j + \in) \qquad (2)$$

where, Z, n_o, n_i, n_{ii} and n_{ij} represent the amount of complex extracted and regression coefficient for intercept, linearity, square and interaction respectively;
x_i, x_j and $\sum$ are the coded process variables and the random errors, respectively.

The model fitness and significance were checked using the coefficient of regression R^2 and

F-test and *P*-value, using mean 3 replicate determinations. Analysis of variance (ANOVA) was used to evaluate the process variables interactions with model fitting to get optimum conditions on a 95% confidence level fixed probability [16].

<div align="right">Table 1</div>

Variables and experimental design levels for response surface methodology (Coded and Uncoded Form).

Coded values	-1.000	0.000	+1.000
A (Seconds)	1.00	8.00	15.00
B (M)	0.50	0.25	0.0001
C (°C)	20.00	30.00	40.00
D (µg/mL)	5.00	12.50	20.00
E (%)	0.05	0.525	1.00

A- extraction time;
B- acid concentration;
C- temperature;
D- metal concentration;
E- ligand concentration.

Synthesis of metal complexes

Sample solution containing 100 µg/mL each of the metal ions Co(II) and Cu(II) were transferred into a 50 mL calibrated extraction bottle. The volume was made up to 5 mL with 0.0001 M HCl, HNO₃, H₂SO₄ or HClO₄ aqueous acid solution. Following, 0.5 mL of 0.5% H₂BMPDE solution was added and the colour was allowed to develop during the maximum time previously determined. This was followed by phase centrifugation and separation of the coloured metal complexes. The complexes were allowed to dry and re-crystallization was performed using carbon tetrachloride (*d.p.*= 300°C).

Characterization

H₂BMPDE (C₁₆H₁₆N₂O₂, ligand): molecular weight: 268 g/mol; yellow crystals; yield 64.45%; *m.p.* 127±1°C; electronic spectrum (DMF, nm, ε= mol⁻¹dm³cm⁻¹) 260 (ε= 3.0x10²), 285 (ε= 3.6x10²) 335 (ε= 4.7x10²). Elemental analysis: calc. C, 71.64 %; H, 5.97%; N, 10.44%; found: C, 70.89%; H, 6.05%; N, 10.41%. FTIR: 3401 cm⁻¹ ν(O-H) aromatic, 3042 cm⁻¹ ν(C-H) aromatic, 2913 cm⁻¹ ν(C-H) aliphatic, 1615 cm⁻¹ ν(C=N) iminic, 1285 cm⁻¹ ν(C-O) phenolic; ¹H-NMR (ppm): 7.4 (1H, N=C(H) methine protons of azomethine); 7.11 (3H, hydrogen of aromatic ring); 6.5 (1H, N-H protons); 3.5 (4H, =NCH₂CH₂N= methylene protons). ¹³C-NMR (ppm): 221.44 (bonded to phenolic oxygen); 152.65 (aromatic carbons); 48.69 (methylene carbon); 3.90 (4H, =NCH₂CH₂N= methine or methylene protons).

Cu(II)HBMPDECl (C₁₆H₁₅N₂O₂ClCu, paramagnetic): molecular weight 366 g/mol; green crystals; yield 40%; *d.p.*= >300°C. Elemental analysis: calc. C, 51.75%; H, 5.028%; N, 10.52%; found: C, 52.45%; H, 4.09%; N, 7.69%. FTIR: 3406 cm⁻¹ ν(OH) aromatic, 3046 cm⁻¹ ν(C-H) aliphatic, 2925 cm⁻¹ ν(N-H), 1933 cm⁻¹ ν(C=O), 1631 cm⁻¹ ν(C=N) iminic, 1414 cm⁻¹ ν(C-O) phenolic, 448 cm⁻¹ ν(Cu-N), 744 cm⁻¹ ν(Cu-O). Electronic spectrum (DMF, nm, ε=mol⁻¹ dm³cm⁻¹), 260 (ε= 7.70x10³), 300 (ε= 3.36x10⁴), 350 (ε= 3.90 x 10⁴); μ_{eff} =1.8 BM.

Co(III)HBMPDE(OH)₂ (C₁₆H₁₅N₂O₂(OH)₂Co, diamagnetic): molecular weight 357.69 g/mol; light green crystals; yield: 45%; *d.p.*=>300°C. Elemental analysis: calc. C, 60.06%; H, 5.631%; N, 10.12%; found: C, 53.67%; H, 5.03%; N, 7.83%. FTIR: 1915 cm⁻¹ ν(C=O) aromatic, 2924 cm⁻¹ ν(N-H), 1623 cm⁻¹ ν(C=N) iminic, 1289 cm⁻¹ ν(C-O) phenolic, 428 cm⁻¹ ν(Co-N), 752 cm⁻¹ ν(Co-O). ¹H-NMR (ppm): 8.14 (1H, N=C(H) methine protons of azomethine); 7.33-7.28 (3H, hydrogen of aromatic ring); 6.84-6.88 (1H, N-H protons); 3.98-4.89 (4H, =NCH₂CH₂N= methylene protons). ¹³C-NMR (ppm): 116.74 (aromatic carbons); 103.53 (aromatic carbons); 68.45, 64.87 (methylene carbon). Electronic spectrum (DMF, nm, ε= mol⁻¹ dm³cm⁻¹) 260 (ε= 1.40 x10³), 335 (ε= 2.3 x 10³), 400 (ε= 2.5 x 10³).

Antibacterial screening

The bio-efficiency of the ligand and Cu(II) and Co(III) complexes was tested using four bacteria (*Staphylococcus aureus, Klebsiella specie, Escherichia coli and Pseudomonas aeuroginosa*) according to the agar well diffusion method as described elsewhere [15]. The zones of microbial growth inhibition around the walls of the H₂BMPDE and metal complexes were examined, measured and recorded in millimetres. The activity index (%) was calculated by Eq.(3):

$$Activity\ Index\ (\%) = \frac{A}{B}100\% \qquad (3)$$

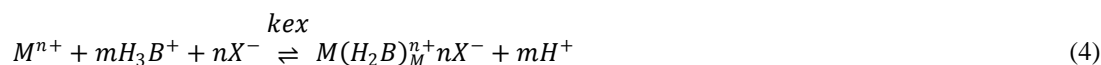where, *A*- zone of inhibition of H₂BMPDE and metal complexes;
B- zone of inhibition of ampicillin used as standard [17].

Results and discussion
Synthesis and characterization

The extraction mechanism of H₂BMPDE-metal complexes from the mineral acids at the studied acid concentrations (0.1-0.0001 M) suggests an anion association process as the ligand was protonated at low pH (10⁻⁴ M) and

could form a complex with the metal cations as a neutral molecule [18]. Consequently, the extraction of the metal (Co(III) and Cu(II)) complexes could be explained using Eqs.(4-6). This suggests t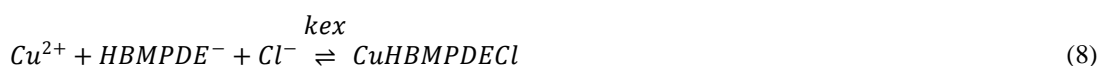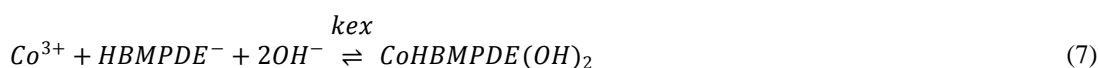he formation of ion-association complexes of the type $[M(HB)^+]X^-$ and $[M(H_2B)^+]2X^-$ [18]. In other words, the extraction mechanism of H_2BMPDE-metal complex formation can be represented as Eqs.(7-8), for Co(III) and Cu(II) respectively.

$$M^{n+} + mH_3B^+ + nX^- \overset{kex}{\rightleftharpoons} M(H_2B)_M^{n+} nX^- + mH^+ \tag{4}$$

$$kex = \frac{[M(H_2B)_M^{n+} \cdot nX^-][H^+]^m}{[M^{n+}][H_3B^+]^m[X^-]^n} \tag{5}$$

where, $X = Cl^-, ClO_4^-, NO_3^-, or HSO_4^-$;

$\quad\quad$ M= metal cation;

$\quad\quad$ D= distribution ratio.

$$log\ D = M\ log\ [H_2B] + n\ log\ [X^-] + m\ log\ K_1 + log\ kex \tag{6}$$

$$Co^{3+} + HBMPDE^- + 2OH^- \overset{kex}{\rightleftharpoons} CoHBMPDE(OH)_2 \tag{7}$$

$$Cu^{2+} + HBMPDE^- + Cl^- \overset{kex}{\rightleftharpoons} CuHBMPDECl \tag{8}$$

The electronic spectrum of the Co(III) complex consists of three bands at 400, 335 and 260 nm. The appearance of the band at 400 nm assignable to $4A_{2g}{\rightarrow}2T_{1g}(F)$ indicated distorted octahedral geometry around the Co(III) ion whereas the bands at 335 and 260 nm formed due to charge transfer transitions [20]. Similarly, the broad band region of the electronic spectra of Co(III) covering the long wavelength region 335-400 nm was assigned to a distorted octahedral $^3A_{2g}{\rightarrow}^2T_{1g}(P)$ transition [20]. The Cu(II) complex has a band displayed in the electronic spectrum in the region of 350 nm typical for $2B_{1g}{\rightarrow}2E_g$ transitions of a distorted square planar geometry. The $2E_g$ and $2B_{1g}$ states of d^9 Cu(II) ion splits up under the influence of the field (tetragonal distortion). The distortion can cause three transitions $2E_g{\rightarrow}2A_{1g}$, $2B_{1g}{\rightarrow}2B_{2g}$ and $2B_{1g}{\rightarrow}2E_g$ typical for distorted square planar geometry [21-22].

The FTIR spectra of the complexes were interpreted by comparing them to the spectra of the free ligand. The IR spectra of the ligand showed a broad band at 3401 cm^{-1} due to the stretching vibrations of phenolic hydroxyl group [22]. The broad band was formed due to intermolecular hydrogen bonding between the azomethine group and the phenolic group [23]. This peak also appeared in the spectra of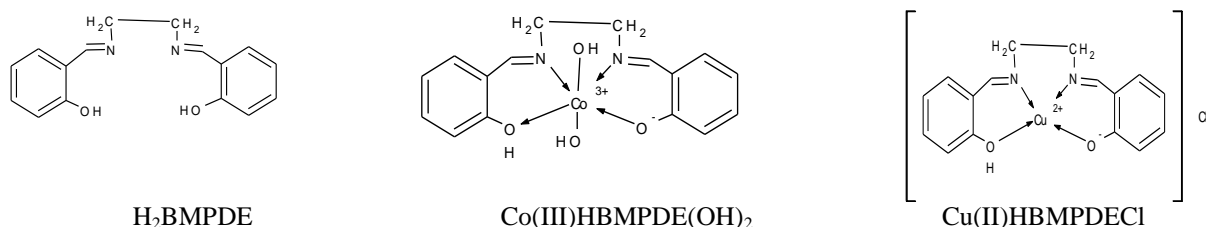 the complexes, due to deprotonation of only one of the salicylideneimine moiety during complex formation. The strong band observed at 1615 cm^{-1} was assigned to the stretching vibrations of the (C=N) azomethine group of the ligand [25]. On chelation, the C=N stretching frequencies of the complexes were slightly shifted to higher frequencies (8-16 cm^{-1}) suggesting that the ligands coordinated with the metal ion through the N_2O_2 donor atom set of the tetradentate Schiff base [23]. This observation was also in agreement with the assertion [23] that C=N stretching frequencies of a complex increase if a Schiff base ligand coordinates to the metal ion as a neutral molecule. The appearance of new bands at 448 cm^{-1} v(Cu-N), 744 cm^{-1} v(Cu-O) and 428 cm^{-1} v(Co-N), 752 cm^{-1} v(Co-O) confirmed the ligation of the metals to the ligand, as they were absent in the spectra of the ligand.

The ^{1}H-NMR spectrum of H_2BMPDE displayed the hydrogen of aromatic rings as complex multiplets at 7.11 ppm (3H) due to coupling of 4 hydrogen atoms in the ring whereas the methine proton of the ethylene bridge was observed as doublet at 3.5 ppm (4H) illustrating the symmetrical nature of the ligand. The appearance of a triplet peak at 6.5 ppm (2H) was assigned to the N-H proton. The azomethine proton was displayed as quartet (1H) at 7.4 ppm [25]. In the ^{1}H-NMR spectrum of Co(III)HBMPDE(OH)$_2$ there were peaks at

8.58 (1H), 3.98 (1H) and 4.89 ppm (1H) as singlets showing methine protons of the azomethine (N=C-H) (8.58 ppm) and the methine proton of the ethylene bridge (3.98 and 4.89 ppm). We observed a down field shift of the signals at the methine proton of 1.14 ppm between the ligand and the complex as a result of complexation through the imine [26]. The signals typical to aromatic protons were observed as multiplets between 7.28-7.33 ppm (2H), whereas the N-H proton was observed as triplet between 6.84-6.88 ppm (1H) [25].

The ^{13}C-NMR spectrum of H$_2$BMPDE showed peaks at 221.44 ppm, 152.65 ppm and 48.69 ppm due to the quartenary carbon atom bonded to the oxygen of phenolic group (-C-O),

the imine bonded carbon (-N=C-H) and the methylene carbon (-NCH$_2$CH$_2$N-), respectively [21]. In the ^{13}C-NMR spectrum of Co(III)HBMPDE(OH)$_2$ complex there were peaks at 116.81, 103.57, 68.45, 64.87, 79.64, 76.08, 74.04 and 73.31 ppm; the peak at 116.81 ppm was assigned to the quaternary carbon atom bonded to imine (-C=N), the peak at 103.57 ppm was assigned to aromatic carbons (C-H), the peaks at 68.45 and 64.87 ppm were assigned to methylene carbons.

The magnetic moment of 1.8 BM, observed for Cu(II)HBMPDECl complex indicated the mononuclear nature of the complex. The structure of the ligand and proposed structures of the complexes are presented in Scheme 2.

H$_2$BMPDE   Co(III)HBMPDE(OH)$_2$ Cu(II)HBMPDECl

Scheme 2. The structure of the ligand (H$_2$BMPDE), and proposed structures of Co(III)HBMPDE(OH)$_2$ and Cu(II)HBMPDECl complexes.

Model fitting and statistical analysis

The experimental values of the yield of the Co(III)HBMPDE(OH)$_2$ and Cu(II)HBMPDECl complexes at each point based on Box-Behnken experimental design are illustrated in Table 1. The obtained data were used to calculate the coefficients of the quadratic second order polynomial Eq.(2), used to predict the yield of the complex. The best fitting model was developed through regression analysis using experimental data. The regression terms, which were determined to be significant, were combined into a fitted second order polynomial equation to predict the yield of Cu(II) and Co(III) complexes as shown in Eq.(9) and Eq.(10), respectively:

Cu(II)Yield = 0.047+0.82A-2.15B-0.13C+4.16D
+0.61E-0.68AB-0.52AC+0.28AD-0.66AE
+0.83BC+1.29BD-0.42B-0.53CD-0.69CE-   (9)
0.67DE+9.51A^2+6.46B^2+9.15C^2+5.74D^2
+9.35E^2

Co(III)Yield = 2.38 +3.22A -2.34B+0.56C
+3.01D+0.22E+0.28AB+0.80AC-2.78AD
+0.000AE+0.28BC+2.83BD-5.40BE   (10)
+0.025CD-5.83CE+3.88DE+8.84A^2
+0.92B^2+8.66C^2+6.55D^2+4.22E^2

F- and *P*-values show the model significance from regression analysis and analysis of variance

(ANOVA). Generally, the decrease of *P* value indicates the increase of model significance [5]. The value of the coefficient of determination (R^2= 0.9734 for Cu(II)HBMPDECl (µg/mL) and R^2= 0.9999 for Co(III)HBMPDE(OH)$_2$ (µg/mL), for the response $P \leq 0.05$) indicated that predicted values and actual values were in agreement and explained the results of 97.34% and 99.99% for Cu(II)HBMPDECl (µg/mL) and Co(III)HBMPDE(OH)$_2$ (µg/mL) [27]. Adjusted R^2 and predicted R^2 differed from each other by 0.0316 and 0.0001 for Cu(II)HBMPDECl (µg/mL) and Co(III)HBMPDE(OH)$_2$ (µg/mL), respectively, indicating strong model agreement. The non-significant lack of fit for *F*-values of 3.63 and 2.43 for Cu(II)HBMPDECl (µg/mL) and Co(III)HBMPDE(OH)$_2$ (µg/mL) further, showed the significance of the predicted model. Consequently, at *P*> 0.05, there was no significant lack of fit. The *F*– values of $1.68 \cdot 10^5$ and $0.08 \cdot 10^5$ for Cu(II)HBMPDECl (µg/mL) and Co(III)HBMPDE(OH)$_2$ (µg/mL) further illustrated the significance of the model. The values of adjusted R^2 of 0.9418 and 0.9997, for the complexes Cu(II)HBMPDECl (µg/mL) and Co(III)H$_2$BMPDE (µg/mL), showed that only 0.316% and 0.001% of the entire variation was not explained [5].

Validation of the predicted model

The optimal conditions for the complexes synthesis such as: time of extraction, acid concentration, temperature, metal concentration and ligand concentration were generated.

Table 2 shows the yields of complexes predicted using the design expert software, which corresponded to 31.89±0.11 µg/mL and 34.50±0.02 µg/mL for Co(III) and Cu(II), respectively. Comparison of predicted values with the experimental values of 30 µg/mL and 32 µg/mL for Co(III) and Cu(II), respectively, indicates very close results as shown in Figures 1 and 2. Thus we can conclude that the system modelling is adequate and appropriate for the optimization of the process. The response surface (*RS*) plots showing the effects of various factor combinations on the complexes yields are summarized in Figures 3-10. As shown, the formation of Cu(II) and Co(III) complexes requires the interaction of all the factors.

Table 2

Optimal conditions for the extraction of Co(III)HBMPDE(OH)$_2$ and Cu(II)HBMPDECl complexes.

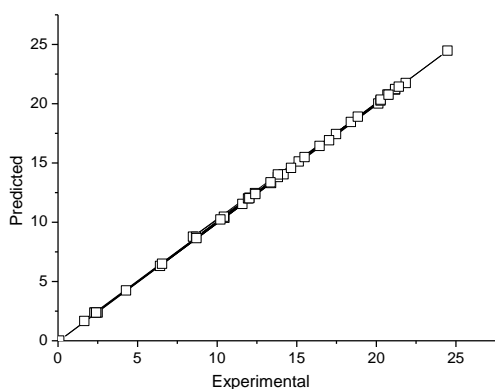| Factors	Factor name	Co(III)	Cu(II)
A	Time (min)	14.83	14.63
B	Acid concentration (M)	0.0001	0.0001
C	Temperature (°C)	39.27	34.22
D	Metal concentration (µg/mL)	6.22	19.99
E	Ligand concentration (%)	0.30	0.10
Predicted yield		31.89±0.11	34.50±0.02

Figure 1. Comparison of predicted and experimental values for Co(III)HBMPDE(OH)$_2$ complex.
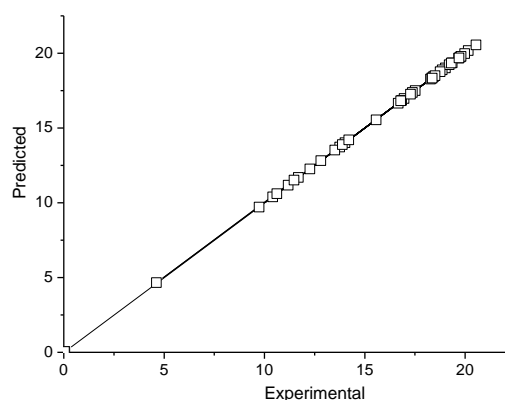

Figure 2. Comparison of predicted and experimental values for Cu(II)HBMPDECl complex.
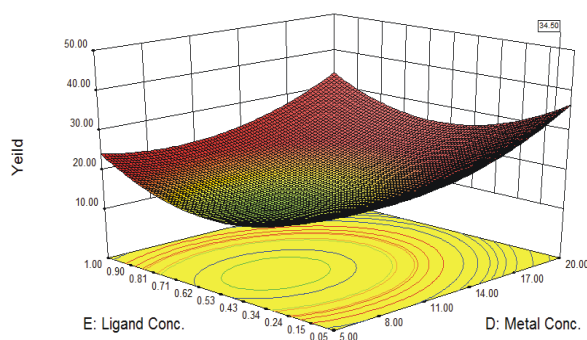

Figure 3. RS plot of metal and ligand concentration for Cu(II)HBMPDECl complex.
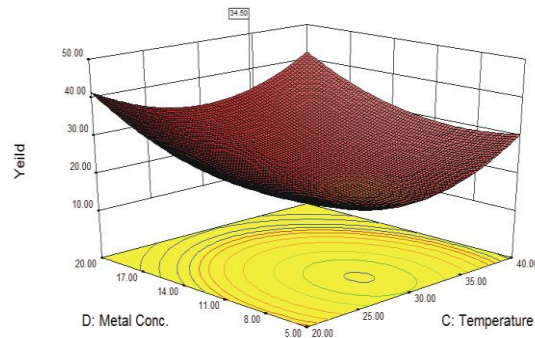

Figure 4. RS plot of metal concentration and temperature for Cu(II)HBMPDECl complex.
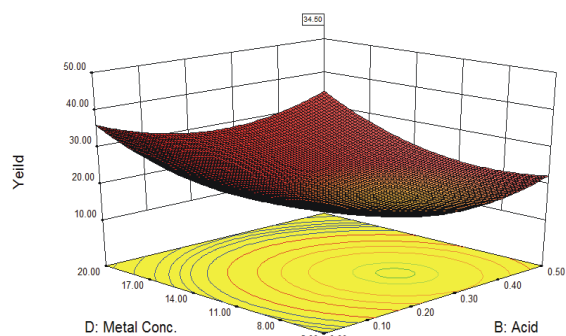
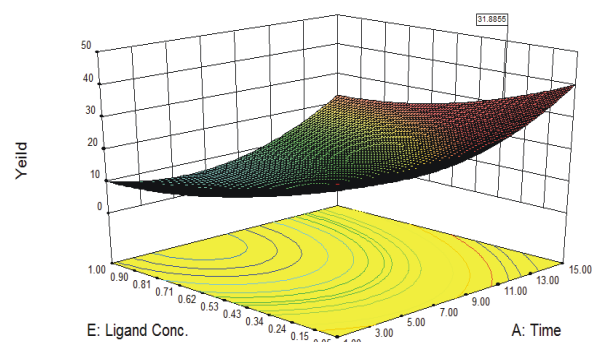**Figure 5. RS plot of metal concentration and acid concentration for Cu(II)HBMPDECl complex.**

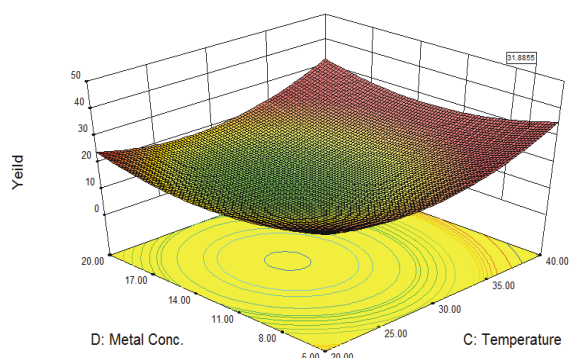**Figure 6. RS of ligand concentration and time for Cu(II)HBMPDECl complex.**

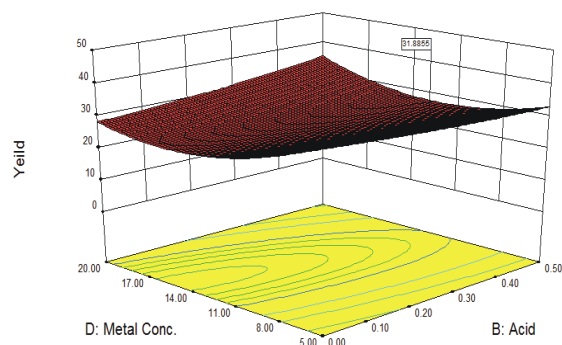**Figure 7. RS plot of metal concentration and temperature for Co(III)HBMPDE(OH)$_2$ complex.**

Figure 8. RS plot of metal concentration and acid concentration for Co(III)HBMPDE(OH)$_2$ complex.
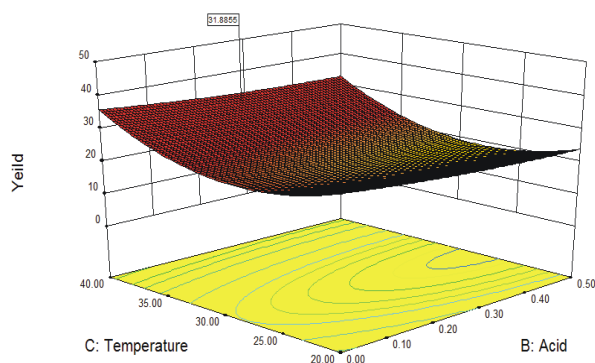

Figure 9. RS of acid concentration and temperature for Co(III)HBMPDE(OH)$_2$ complex.
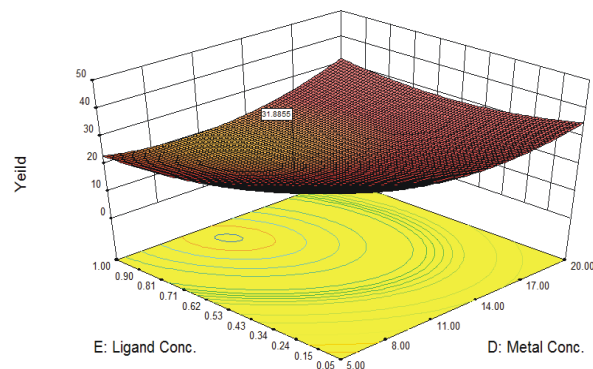

Figure 10. RS plot of ligand concentration and metal concentration for Co(III)HBMPDE(OH)$_2$ complex.

Screening of antibacterial activity

It was observed that metal complexes have higher activity than the ligand, as shown in Table 3. This is probably due to chelation, which facilitates the ability of a complex to cross the microorganism's cell membrane [21]. The metal complexes were prepared using different acid solutions and the antibacterial effect was investigated to assess if the presence of the anions attached to the metal complexes influenced the bio-efficiency.

The complex Co(III)HBMPDEX prepared using HCl solution has the highest activity index of 114.3% for *Pseudomonas aeuroginosa* followed by Co(III)HBMPDEX prepared using HNO$_3$ solution with an activity index of 113.63% for *Klebsiella specie*. No general trend was observed in the antibacterial assessment of the metal complexes prepared in the various acid solutions but that was observed for a particular bacterial strain as shown in Table 3.

Table 3

Activity index of H₂BMPDE and Cu(II) and Co(III) complexes in comparison to standard ampicillin.

H₂BMPDE, Metal complex (acid solution)	Activity Index (%)			
	Pseudomonas aeuroginosa	*Klebsiella specie*	*Escherichia coli*	*Staphylococcus aureus*
Co(III)HBMPDEX (H₂SO₄)	47.60	27.27	17.65	28.57
Co(III)HBMPDEX (HCl)	114.30	31.82	35.29	-
Co(III)HBMPDEX (HNO₃)	66.67	113.63	41.17	-
Cu(II)HBMPDEX (H₂SO₄)	57.14	45.45	27.27	76.19
Cu(II)HBMPDEX (HCl)	47.60	45.45	70.59	57.14
Cu(II)HBMPDEX (HNO₃)	38.09	54.55	29.41	95.23
H₂BMPDE	-	18.18	17.65	28.57

Conclusions

The complexes Co(III)HBMPDE(OH)₂ and Cu(II)HBMPDECl were synthesized using the extractive technique. The 1:1 molar ratio of metal:ligand complexes, of distorted octahedral and distorted square planar geometries, were extracted from aqueous solutions into chloroform phase, re-crystallized and dried. The ion associated metal complexes were physico-chemically characterized and the results indicated that Co(III)HBMPDE(OH)₂ was diamagnetic while Cu(II)HBMPDECl was paramagnetic. Response surface methodology and Box-Behnken design approach were used to model the preparation and extraction of the metal complexes. The calculated optimal conditions for the synthesis and extraction of the metal complexes allow designing the synthesis at industrial scale.

The ligand and the metal complexes prepared in different mineral acids are active against both gram positive and gram negative bacteria, Co(III)HBMPDEX-(HCl) having the highest activity index for *Pseudomonas aeuroginosa,* followed by Co(III)HBMPDEX-(HNO₃) for *Klebsiella specie*.

References

1. Hu, X.M.; Xue, L.W.; Zhao, G.Q.; Yang, W.C. Synthesis, crystal structure and antimicrobial activity of a hetero trinuclear manganese(III)-iron(II) complex derived from N,N'–bis(5–methylsalicylidine)-1,2–diaminoethane. Bulletin of the Chemical Society Ethiopia, 2015, 29(3), pp. 407-413.
DOI: http://dx.doi.org/10.4314/bcse.v29i3.8
2. Yang S.; Kou, H.; Wang, H.; Cheng, K.; Wang, J. Efficient electrolyte of N,N'-bis (salicylidene)ethylenediamine zinc (II) iodide in dye-sensitized solar cells. New Journal of Chemistry, 2010, 34, pp. 313-317.
DOI: 10.1039/B9NJ00405J.
3. Dardfarnia, S.; Haji Shabani, A.M.; Kazemi, E.; Khormizi, S.A.H.; Tammadon, F. Synthesis of nano-pore size Ag(I)-imprinted polymer for the extraction and preconcentration of silver ions followed by its determination with flame atomic absorption spectrometry and spectrophotometry using localized surface plasmon resonance peak of silver nanoparticles. Journal of the Brazilian Chemical Society, 2015, 26(6), pp. 1180-1190.
DOI: https://dx.doi.org/10.5935/0103-5053.20150082.
4. Cozzi, P.G. Metal –salen Schiff base complexes in catalysis: Practical aspects. Chemical Society Review, 2004, 33(7), pp. 410-421.
DOI: https://doi.org/10.1039/b307853c.
5. Ferreira, S.L.C.; Bruns, R.E.; Paranhos da Silva, E.G.; Lopes dos Santos, W.N.; Quintella, C.M.; David, J.M.; Bittencourt de Andrade, J.; Breitkreitz, M.C.; Jardim, I.C.S.F.; Neto, B.B. Statistical designs and response surface techniques for the optimization of chromatographic systems. Journal of Chromatography A, 2007, 1158(1-2), pp. 2-14.
DOI: https://doi.org/10.1016/j.chroma.2007.03.051.
6. Woldemariam, G.A.; Mandal, S.S. Iron (III)-salen damages DNA and induces apoptosis in human cell *via* mitochondrial pathway. Journal of Inorganic Biochemistry, 2008, 102(4), pp. 740-747. DOI: http://dx.doi.org/10.1016/j.jinorgbio.2007.11.008.
7. Feng, X.; Zhang, T.; Chen, J.L.; Huo, S.Z.; Luo, G.U. One-pot synthesis, crystal structure and fluorescence property of a Cd (II) complex based on monosalicylaldehyde Schiff base ligand. Russian Journal of Coordination Chemistry, 2014, 40(7), pp. 510-514.
DOI: https://doi.org/10.1134/S1070328414070033.
8. Doctrow, S.R.; Huffman, K.; Marcus, C.B.; Tocco, G.; Malfroy, E.; Adinolfi, C.A.; Kruk, H.; Baker, K.; Lazarowych, N.; Mascarenhas, J.; Malfroy, B. Salen-manganese complexes as catalytic scavengers of hydrogen peroxide and cytoprotective agents: structure-activity relationship studies. Journal of Medicinal Chemistry, 2002, 45(20), pp. 4549-4558.
DOI: 10.1021/jm020207y.
9. Ganjali, M.R.; Poursaberi, T.; Basiripour, F.; Salavati-Niassari, M.; Yousefi, M.; Shamsipur, M.

Highly selective thiocyanatepolyl(vinylchloride) membrane electrode based on a cadmium-Schiff's base complex. Fresenius Journal of Analytical Chemistry, 2001, 370(8), pp. 1091-1095. DOI: https://doi.org/10.1007/s002160100.

10. Hambley, T.W. Metal-Based Therapeutics. Science, 2007, 318(5855), pp.1392-1393. DOI: 10.1126/science.1150504.

11. Farrer, N.J.; Salassa, L.; Sadler, P.J. Photoactivated chemotherapy (PACT): the potentials of excited – state d- block metals in medicine. Daltons Transactions, 2009, 0(48), pp. 10690-10701. DOI: 10.1039/B917753A.

12. Karuppasamy, P.; Thiruppathi, J.; Vijaya Sundar, J.; Rajapandian, V.; Ganesan, M.; Rajendran, T.; Rajapal, S.; Nagarajan, N.; Rajendran, P.; Sivasubramanian, V.K. Spectral, computational, electrochemical and antibacterial studies of iron (III)-salen complexes. Arabian Journal for Science and Engineering, 2015, 40(10), pp. 2945-2958. DOI: https://doi.org/10.1007/s13369-015-1599-8.

13. Prakash, A.; Pal Gangwar, M.; Singh, K.K. Synthesis, spectroscopy and biological studies of Ni(II) complexes with tetradentate Schiff bases having N_2O_2 donor group. Journal of Developmental Biology and Tissue Engineering, 2011, 3(2), pp. 13-19.

14. Golcu, A.; Tumer, M.; Demirelli, H.; Wheatley, R.A. Cd (II) and Cu(II) complexes of polydentate Schiff base ligands: synthesis, characterization, properties and biological activity. Inorganica Chimica Acta, 2005, 358(6), pp. 1785-1797. DOI: https://doi.org/10.1016/j.ica.2004.11.026.

15. Musthak, A.; Mallikarjuna Rao, R.; Rafi, M.; Jaffer Mohiddin, G.; Sreeramulu, J. Synthesis, spectral analysis and antimicrobial activity of novel dithiocarbamate Schiff base metal complexes. Archives of Applied Science Research, 2012, 4(2), pp. 858-862.

16. Thakur, A.; Panesar, P.S.; Saini, M.S. Response surface modeling of lactic acid extraction by emulsion liquid membrane: box-Behnken experimental design. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 2014, 8(8), pp. 873-881.

17. Ukoha, P.O.; Nwabue, F.I.; Obasi, L.N. Solvent extraction studies on copper (II) and silver(I) complexes of bis(4-hydroxypent-2-ylidene)diaminoethane: Composition of extracted copper(II) species. E-journal of Chemistry, 2011, 8(4), pp. 1864-1871. DOI: http://dx.doi.org/10.1155/2011/582649.

18. Abd El-Wahab, Z.H.; Mashaly, M.M.; Salman, A.A.; El-Shetary, B.A.; Faheim, A.A. Co(II), Ce(III) and UO_2(VI)bis-salicylatothiosemicabazide complexes: binary and ternary complexes, thermal studies and antimicrobial activity. Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy, 2004, 60(12), pp. 2861-2873.

DOI: https://doi.org/10.1016/j.saa.2004.01.021.

19. El-Gamel, N.E.A.; Zayed, M.A. Synthesis, structural characterization and antimicrobial activity evaluation of metal complexes of sparfloxacin. Spectrochimica Acta: Part A, Molecular and Biomolecular Spectroscopy, 2011, 82(1), pp. 414-423. DOI: https://doi.org/10.1016/j.saa.2011.07.072.

20. Zayed, M.A.; Nour El-Dien, F.A.; Mohamed, G.G.; El-Gamel, N.E.A. Synthesis and thermal characterization of new ternary chelates of piroxicam and tenoxicam with glycine and DL-phenylalanine and some transition metals. Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy, 2006, 64(1), pp. 216-232. DOI: https://doi.org/10.1016/j.saa.2005.06.042.

21. Faniran, J.A.; Patel, K.S.; Bailar Jr, J.C. Infrared spectra of N,N′-bis(salicylidene)-1,1-(dimethyl)ethylenediamine and its metal complexes. Journal of Inorganic and Nuclear Chemistry, 1974, 36(7), pp. 1547-1551. DOI: https://doi.org/10.1016/0022-1902(74)80621-4.

22. Thangadurai, T.D.; Natarajan, K. Mixed ligand complexes of ruthenium (II) containing α,β- unsaturated-β-ketoamines and their antibacterial activity. Transition Metal Chemistry, 2001, 26(4-5), pp. 500-504. DOI: https://doi.org/10.1023/A:101109951.

23. Aranha, P.E.; dos Santos, M.P.; Romera, S.; Dockal, E.R. Synthesis, characterization and spectroscopic studies of tetradentate Schiff base chromium (III) complexes. Polyhedron, 2007, 26(7), pp. 1373-1382. DOI: https://doi.org/10.1016/j.poly.2006.11.005.

24. Thompson, K.H.; Orvig, C. Metal complexes in medicinal chemistry: New vistas and challenges in drug design. Dalton Transaction, 2006, 0(6), pp. 761-764. DOI: https://doi.org/10.1039/B513476E.

25. Emara, A.A.A.; Ali, A.M.; El-Asmy, A.F.; Ragab, E.M. Investigation of the oxygen affinity of manganese(II), cobalt(II) and nickel(II) complexes with some tetradentate Schiff bases. Journal of Saudi Chemical Society, 2014, 18(6), pp. 762-773. DOI: http://doi.org/10.1016/j.jscs.2011.08.002.

26. Natarajan, R.; Sivasangu, S.; Mitu, L. Synthesis, structure elucidation, DNA interaction, biological evaluation and molecular docking of an isatin-derived tyramine bidentate Schiff base and its metal complexes. Monatshefte für Chemie - Chemical Monthly, 2012, 143(7), pp. 1019-1030. DOI: https://doi.org/10.1007/s00706-011-0699-8.

27. Ravikumar, K.; Pashirajan, K.; Swaminathan, T.; Balu, K. Optimization of batch process parameters using response surface methodology for dye removal by a novel adsorbent. Chemical Engineering Journal, 2005, 105(3), pp. 131-138. DOI: https://doi.org/10.1016/j.cej.2004.10.008.