

# Binding of Carbonated Anions to Cationic Hexaureachromium(III): Synthesis, Characterization and Antimicrobial Activity of $[\text{Cr}(\text{CO}(\text{NH}_2)_2)_6](\text{HCO}_3)_3$ and $[\text{Cr}(\text{CO}(\text{NH}_2)_2)_6]_2(\text{CO}_3)_3$

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## Abstract

**Aim:** The aim of the study is to synthesize two new *hexaureachromium* complexes:  $[\text{Cr}(\text{CO}(\text{NH}_2)_2)_6](\text{HCO}_3)_3$  (1),  $[\text{Cr}(\text{CO}(\text{NH}_2)_2)_6]_2(\text{CO}_3)_3$  (2) followed by the analysis of binding ability of hexaureachromium(III) cation towards carbonated anions ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) and their antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* *S. aures* (for anti-bacterial), and *Aspergillus niger* (for anti-fungal). **Materials and Methods:** The synthesized compounds **1** and **2** were characterized by spectroscopic studies (fourier-transform infrared and ultraviolet [UV]-visible). The binding properties of  $[\text{Cr}(\text{CO}(\text{NH}_2)_2)_6]^{3+}$  ( $[\text{HuCr}]^{3+}$ ) with carbonated anions ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) in aqueous medium have been determined with standard UV-visible spectrophotometer through titration method. Further, the stoichiometric ratios for complexes **1** and **2** were found through Job's plot. All compounds were also tested for antimicrobial activity against selected strains. **Results and Discussion:** The cation,  $[\text{HuCr}]^{3+}$ , contains ligand urea bearing H-bond donors which are capable of forming hydrogen bonds. The binding constant values obtained are found to be 10.5334 for and 15.7377 for . Further, the stoichiometric ratios for complexes **1** and **2** have been found to be 1:3 and 2:3, respectively.  $[\text{HuCr}]\text{Cl}_3$ , **1** and **2** show the maximum zone of inhibition at 200  $\mu\text{g}/\text{mL}$  but the starting material  $[\text{HuCr}]\text{Cl}_3$  and compound **1** does not show any activity against *A. niger* at 50 and 100  $\mu\text{g}/\text{mL}$  concentration. **Conclusion:** The ionic reactions between anion receptor  $[\text{HuCr}]^{3+}$  and carbonated anions in aqueous solution have been investigated and characterized by spectroscopic (infrared and UV-visible) techniques. The results obtained indicate that the binding ability of the cation  $[\text{HuCr}]^{3+}$  towards carbonated anions is in the order  $\text{CO}_3^{2-} > \text{HCO}_3^-$ . Thus, the selectivity of receptors for particular anion can be chosen. The results predict that the octahedral geometry of  $[\text{HuCr}]^{3+}$  is capable enough to target both the carbonated anions.  $[\text{HuCr}]\text{Cl}_3$  and compound **1** do not show any activity against *A. niger* at 50 and 100  $\mu\text{g}/\text{mL}$  concentration while compound **2** is found to be potent against all the tested organisms (*E. coli*, *S. aures*, and *A. niger*).

**Key words:** Anion receptor, Carbonated anions, Coordination chemistry, Hexaureachromium(III), Spectroscopy

## INTRODUCTION

Anion receptors are receiving increased attention of analysts in recent years and are adding new thrust in research area of coordination chemistry.<sup>[1,2]</sup> Anion plays innumerable roles in the field of catalysis,<sup>[3]</sup> biology,<sup>[4]</sup> medicines<sup>[5-7]</sup> and to control environmental pollution.<sup>[8-11]</sup> They pose more challenges as compare to corresponding cations because (1) they possess different geometries (halides (spherical), (tetrahedral), (linear) and (octahedral)), (2) their size is larger than cations which make them less efficient for electrostatic

binding to receptors, and (3) pH sensitivity. Hence, to design anion receptors with complex sizes and geometries, a great deal of reciprocity must be taken into consideration for the

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selective binding of the anions. Modern supramolecular chemistry utilizes “hydrogen bonding” in the advancement of receptors for the anions recognition.<sup>[12]</sup> As compare to the relatively simple design principles for cation receptor, there are many more factors which may affect the efficacy of artificial anion receptors. Essential points which are required to be undertaken in the designs are the physical features of the units to be assembled and the interaction through which the units are held.<sup>[13]</sup> Previously, many organic based anion receptors have been outlined, viz. cyclic polyamines,<sup>[14-16]</sup> sapphyrins,<sup>[17]</sup> tripodal amines,<sup>[18]</sup> azacryptands,<sup>[19]</sup> calixarenes,<sup>[20]</sup> and azacage<sup>[21]</sup> etc. but a very little reports are available with inorganic anion receptors specially transition metal complexes except a few complexes such as alkoxide-bridged dinuclear Zn(II),<sup>[22]</sup> urea based Pt(II)<sup>[23]</sup> and bipyridine Ru(II).<sup>[24]</sup>

The complex cation,  $[\text{HuCr}]^{3+}$  not only fulfills the properties of cationic metal-based receptors but also has positive charge (3+), 24 hydrogen donor groups (N-H), and stable Oh geometry (hourglass shape) which may bind the carbonated anions effectively. Moreover,  $[\text{HuCr}]^{3+}$  is also very important as it enhances the glucose tolerance factor in rats and pigs.<sup>[25]</sup>

Thus, in continuation of our research to explore interactions of hexaureachromium(III) complex with a variety of anions,<sup>[26,27]</sup> this article reports two new hexaureachromium complexes,  $[\text{HuCr}](\text{HCO}_3)_3$  and  $[\text{HuCr}]_2(\text{CO}_3)_3$  which have been synthesized and characterized by spectroscopic studies (Fourier-transform infrared [FT-IR] and ultraviolet [UV]-visible). Further, binding properties of cation  $[\text{HuCr}]^{3+}$  with carbonated anions have been studied using standard UV-visible spectroscopic titrations in an aqueous medium.

## MATERIALS AND METHODS

$[\text{HuCr}]\text{Cl}_3 \cdot 3\text{H}_2\text{O}$  was synthesized by standard method.<sup>[28]</sup> Estimation of chromium was carried out by the gravimetric method.<sup>[29]</sup> Solid UV/visible spectra were recorded using Cary 5000 UV-visible NIR Spectrophotometer. For titrations, UV/visible spectra were determined with Shimadzu 1800 at 25 ( $\pm 1$ )°C in thermostate. The absorption spectra were recorded in the range 200–800 nm. Infrared (IR) spectra of the said compounds were determined with Varian Resolution Pro 660 FT-IR Spectrophotometer.

### Synthesis of $[\text{HuCr}](\text{HCO}_3)_3$ (1)

The solutions of 1 g of  $[\text{HuCr}]\text{Cl}_3 \cdot 3\text{H}_2\text{O}$  and 0.486 g of sodium bicarbonate each in 10 mL of distilled water were mixed together which resulted immediately into grayishgreen colored precipitates which were filtered, washed, and air dried. The reaction yield was 78.47%.

### Synthesis of $[\text{HuCr}]_2(\text{CO}_3)_3$ (2)

The solutions of 1 g of  $[\text{HuCr}]\text{Cl}_3 \cdot 3\text{H}_2\text{O}$  and 0.277 g of sodium carbonate each in 10 mL of distilled water were mixed together which resulted immediately into grayishgreen colored precipitates which were filtered, washed, and air dried. The reaction yield was yield is 79.86%.

### Job's plot

Stock solution of  $[\text{HuCr}]\text{Cl}_3$  and the anions ( ) were prepared in aqueous medium in same concentration ( $1 \times 10^{-3}$  M). An aliquot (0,1,2...10 mL) of sodium salt of carbonated anions was added in receptor solution in such a way so that the total concentration of the solution remain  $1 \times 10^{-3}$  M. The composition of receptor to anion varied from 10:0 to 0:10.

The stoichiometric ratios of said solutions were determined with the help of UV-visible spectra and Job's plot (continuous variation method) at  $\lambda_{\text{max}} = 619$  nm for both the complexes.

### UV-visible titrations

The binding capability of  $[\text{HuCr}]^{3+}$  for carbonated anions was determined with the help of UV-visible titrations in double-distilled water. The stock solution of sodium salt of carbonated anions ( $1 \times 10^{-4}$  M) and receptor  $[\text{HuCr}]\text{Cl}_3$  ( $1 \times 10^{-2}$  M) were prepared and their titrations were performed by placing 3 mL solution of sodium salt of carbonated anions in quartz cuvette against addition of 2  $\mu\text{L}$  cation solution. The spectra were recorded after 2 min. of each addition. The data of absorption spectra were fitted in the global analysis program Hypspec2014<sup>[30]</sup> to determine the binding constant.

### Antimicrobial activity

#### Microbial strain and culture conditions

Two strains of bacteria namely *Escherichia coli* (MTCC 452), *Staphylococcus aureus* (MTCC 737), and one strain of pathogenic fungus *Aspergillus niger* (MTCC 1344) were purchased from the Microbial Type Culture Collection (MTCC, IMTECH), Institute of Microbial Technology, Chandigarh, India. All the strains had been stored at freeze temperature (4°C) before use. Nutrient agar (NA) and Potato dextrose agar (PDA) (Hi-media) had been used for the initiation of bacteria and fungus. Inoculation of bacteria had been prepared by developing freeze-dried cells in NA for 24 h at 37°C and fungal strain had been inoculated in PDA for 72 h at 30°C. For positive reference standard, ciprofloxacin and fluconazole (Central Drug House Pvt. Ltd., India) had been used and all the samples were prepared in dimethylsulfoxide (DMSO).

### Preparation of test sample

The starting material ( $[\text{HuCr}]\text{Cl}_3$ ) and synthesized compounds **1** and **2** were dissolved in DMSO to attain different concentrations (50, 100, and 200  $\mu\text{g}/\text{mL}$ ).

### Anti-microbial assays

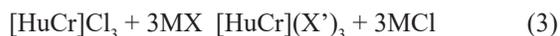
The anti-microbial activity was analyzed by the well diffusion method. 40 mL of NA was used to prepare the Petri plates for the selected bacteria and PDA was used for the preparation of Petri plates for fungus. After that, the selected test organisms were inoculated over the solidified agar plate by using a micropipette, spread it by the spreader and allowed to dry it for 10 min. Three wells were prepared in agar Petri plate by a sterilized steel borer and were finally filled with the standard and solutions of test compounds. All these work were done under aseptic conditions for microbial assay. Bacterial plates were incubated at  $37 \pm 1^\circ\text{C}$  for 24 h and fungal was incubated at  $28 \pm 1^\circ\text{C}$  for 72 h. The anti-microbial potential of the tested compounds were found on the basis of mean diameter of the zone of inhibition around the wells in millimeters (mm). Each assay was done in a triplicate form.

## RESULTS AND DISCUSSION

### Synthesis

#### Compound 1

In aqueous medium hexaureachromium(III) chloride could react with sodium bicarbonate in three possible ways according to their stoichiometric ratios (equations (1-3)). However, the reaction was carried out as per stoichiometry of equation (3).



Where  $\text{M} = \text{Na}^+$ ,  $\text{X}' = \text{HCO}_3^-$

#### Compound 2

In aqueous medium hexaureachromium(III) chloride could react with sodium carbonate in three possible ways according to their stoichiometric ratios (equations (4-6)). However, the reaction was carried out as per stoichiometry of equation (6).



Where  $\text{M} = \text{Na}^+$ ,  $\text{X}'' = \text{CO}_3^{2-}$

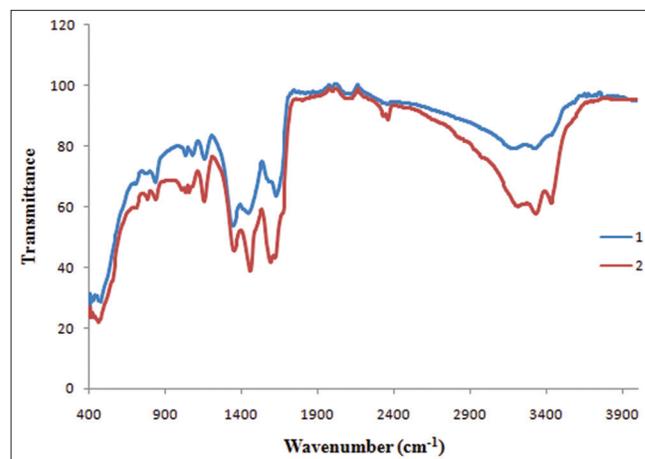
### Infrared spectroscopy

The IR spectrum peaks observed in both the compounds (**1** and **2**) were compared with the peaks observed in case of urea and other similar hexaureachromium(III) compounds.<sup>[28]</sup> For both the complexes, symmetric and asymmetric vibrational modes of  $\text{NH}_2$  group found to be in the range  $3450\text{--}3200\text{ cm}^{-1}$  which appears to be strong and broad alongwith an additional less intense band which attributed to free  $\text{NH}_2$  (non-coordinated). The  $\text{NH}_2$  bending vibration was observed near  $1620\text{ cm}^{-1}$  for both the complexes. The stretching frequency due to  $\nu(\text{CO}) + \delta(\text{NH}_2)$  is observed at  $1595\text{ cm}^{-1}$

**Table 1:** FT-IR band assignments of complexes **1**, **2** and their comparison with  $[\text{HuCr}]\text{Cl}_3$

$\nu$ ( $\text{cm}^{-1}$ )	$[\text{HuCr}]\text{Cl}_3$	<b>1</b>	<b>2</b>
$\nu_a$ ( $\text{NH}_2$ )	3421s	3435b	3429b
$\nu_s$ ( $\text{NH}_2$ )	3295s	3332b	3332b
$\nu$ ( $\text{NH}_2$ free)	3220s	3190b	3210b
$\delta_s$ ( $\text{NH}_2$ )	1617s	1625s	1620s
$\nu$ ( $\text{CO}$ ) + $\delta$ ( $\text{NH}_2$ )	1535s	1595s	1595s
$\nu_a$ ( $\text{CO}_3$ )	-	1446*s	1461*s
$\nu_a$ ( $\text{CN}$ )	1485s	1446*s	1461*s
$\nu_s$ ( $\text{CO}_3$ )	-	1341s	1349s
$\rho_r$ ( $\text{NH}_2$ )	1148m	1155m	1155m
$\nu_s$ ( $\text{CN}$ )	1034m	1080m	1028m
$\delta$ ( $\text{NCO}$ )	626m	692m	695m
$\delta$ ( $\text{NCN}$ )	521m	477m	462m

$\nu_a$  = asymmetric stretch,  $\nu_s$  = symmetric stretch,  $\delta$  = bending,  $\rho$  = rocking,  $\omega$  = wagging, s = strong, m = medium, w = weak, \* = merged peaks. FT-IR: Fourier-transform infrared



**Figure 1:** Fourier-transform infrared spectrum of compound **1** and **2**

for both the complexes. Moreover, two very strong bands for asymmetric and symmetric carbonyl group of carbonate

appeared at 1446 and 1341  $\text{cm}^{-1}$  for compound **1**, 1461 and 1349  $\text{cm}^{-1}$  for compound **2**. The asymmetric CN stretching frequency observed for free urea at 1485  $\text{cm}^{-1}$  shift towards lower frequency in both complexes which also may be due to hydrogen bonding of C-N-H (from cation) with oxygen of carbonyl group and merging of this peak with asymmetric stretching frequency of carbonyl group. The  $\text{NH}_2$  rocking and symmetric CN stretching frequencies were obtained at 1155 and 1080  $\text{cm}^{-1}$  for compound **1** and at 1155 and 1028  $\text{cm}^{-1}$  for compound **2** respectively [Table 1]. The medium absorption bands were observed at 692 and 447  $\text{cm}^{-1}$  for compound **1** and 695 and 462  $\text{cm}^{-1}$  for compound **2** which are assigned to  $\delta(\text{NCO})$  and  $\delta(\text{NCN})$  respectively. All the peaks of compounds **1** and **2** have been assigned in consultation with literature values<sup>[28,31]</sup> and their FT-IR spectra are shown in Figure 1.

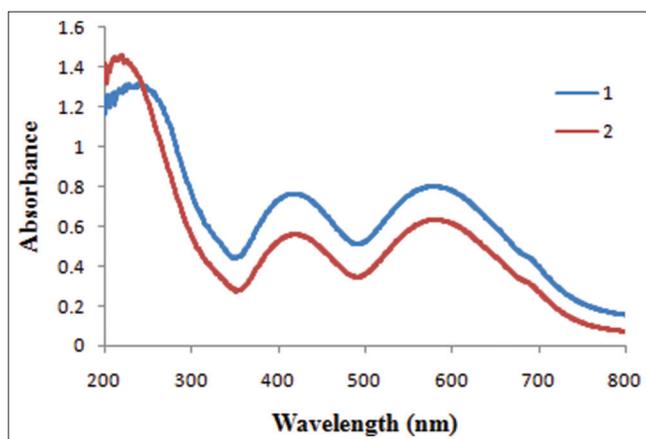


Figure 2: Ultraviolet-visible spectrum of compound **1** and **2**

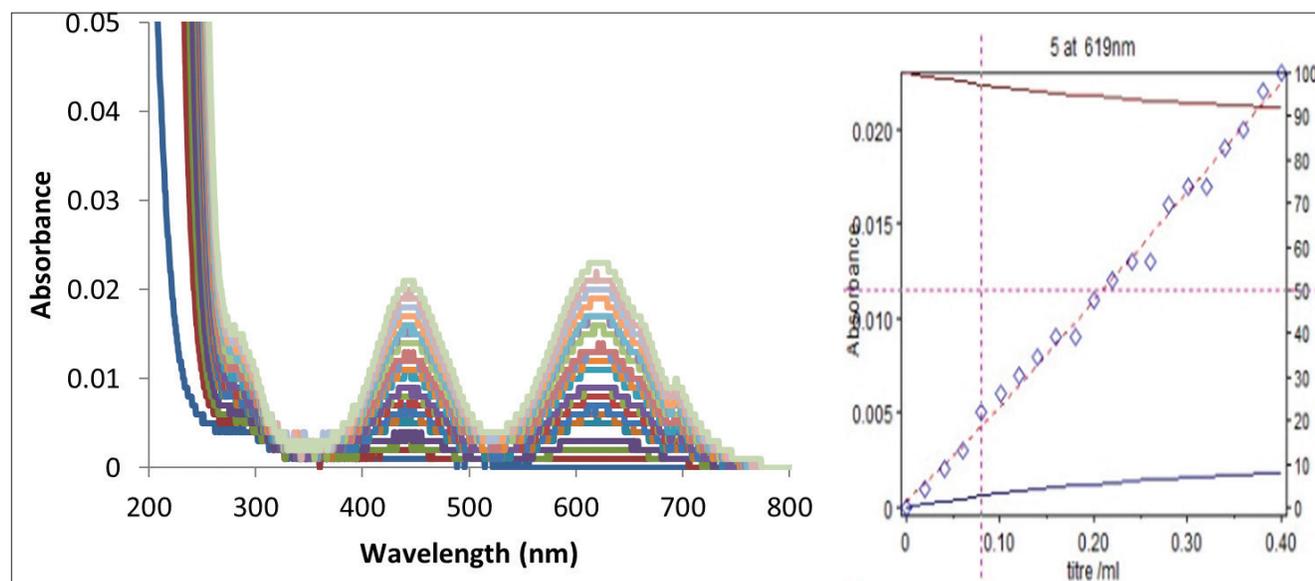


Figure 3: Ultraviolet/visible titration spectra of sodium salt of bicarbonate ( $1 \times 10^{-4}$  M) in aqueous medium after the addition of 2  $\mu\text{L}$  of cation  $[\text{HuCr}]\text{Cl}_3$  ( $1 \times 10^{-2}$  M)

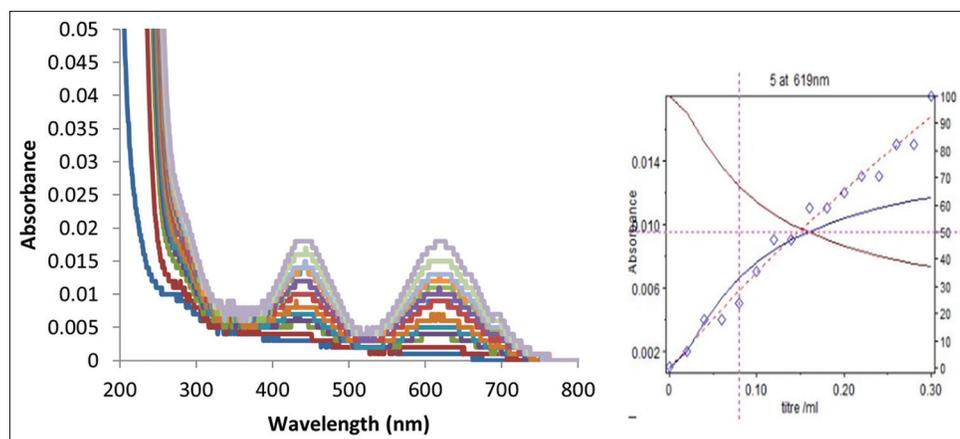


Figure 4: Ultraviolet/visible titration spectra of sodium salt of carbonate ( $1 \times 10^{-4}$  M) in aqueous medium after the addition of 2  $\mu\text{L}$  of cation  $[\text{HuCr}]\text{Cl}_3$  ( $1 \times 10^{-2}$  M)

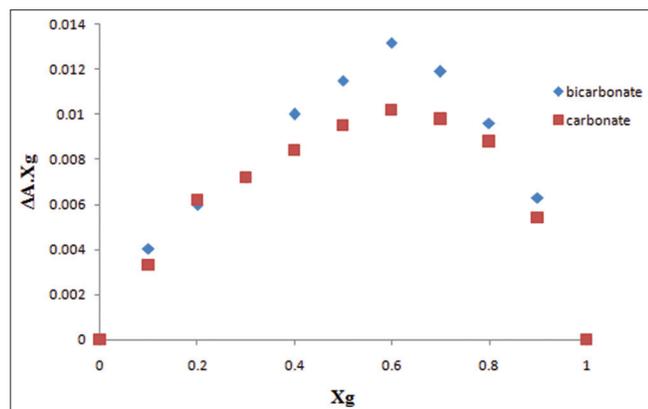
## UV-visible spectroscopy

The UV-Visible spectrum of  $[\text{HuCr}]\text{Cl}_3$  shows purely three d-d transitions  ${}^4\text{A}_{2g} \rightarrow {}^4\text{T}_{2g}(\text{F})$  at 619 nm,  ${}^4\text{A}_{2g} \rightarrow {}^4\text{T}_{1g}(\text{F})$  at 444 nm and  ${}^4\text{A}_{2g} \rightarrow {}^4\text{T}_{1g}(\text{P})$  at 298 nm (sh). The new Cr(III) complexes show three bands at 579, 419, 228 nm in case of **1** and 579, 418, 220 nm in case of **2** as shown in Figure 2.

## Binding properties

For investigating the binding property of  $[\text{HuCr}]^{3+}$  for carbonated anions ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ), the titration experiment was performed by as given in the experimental section. Firstly, the spectrum of the stock solution of sodium salt of carbonated anions ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) has been taken which show only one absorption peak at 265 nm for both the anions. After constant addition of aliquots of 2  $\mu\text{l}$  of  $[\text{HuCr}]\text{Cl}_3$ , the gradual increase in this absorption intensity is observed (hyperchromic shift) which further leads to red shift in bands and found to be at 288 nm in case

of sodium bicarbonate [Figure 3] and 282 nm in case of sodium carbonate [Figure 4]. The red shift in the presence of sodium salt of carbonated anions ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) may result from the interaction between  $[\text{HuCr}]\text{Cl}_3$  and anion. Two new peaks in the range 430–470 nm and 610–630 nm have been generated on addition of cation with a gradual increase in absorption intensity (hyperchromic shift) which further goes to shift in bands as well. The peak observed at 433 and 430 nm in case of bicarbonate and carbonate respectively shifted towards longer wavelength (red shift) and observed at 443 and 441 nm. Peaks at 621 and 616 nm were also observed but there is no shift in wavelength has been observed in case of bicarbonate while there is shift of 4 nm in case of carbonate (621 nm in case of bicarbonate and 620 nm in case of carbonate). The emergence of isobestic points in case of bicarbonate and carbonate were observed at 330 and 347 nm respectively, which shows the complete transfer of free carbonated anion into compound **1** and **2** as shown in Figures 3 and 4. The spectral fitting of the titration data gives values of  $\log \beta$  [Table 2]. The results obtained indicate that the binding ability of the cation  $[\text{HuCr}]^{3+}$  towards carbonated anions is in the order  $\text{CO}_3^{2-} > \text{HCO}_3^-$ .

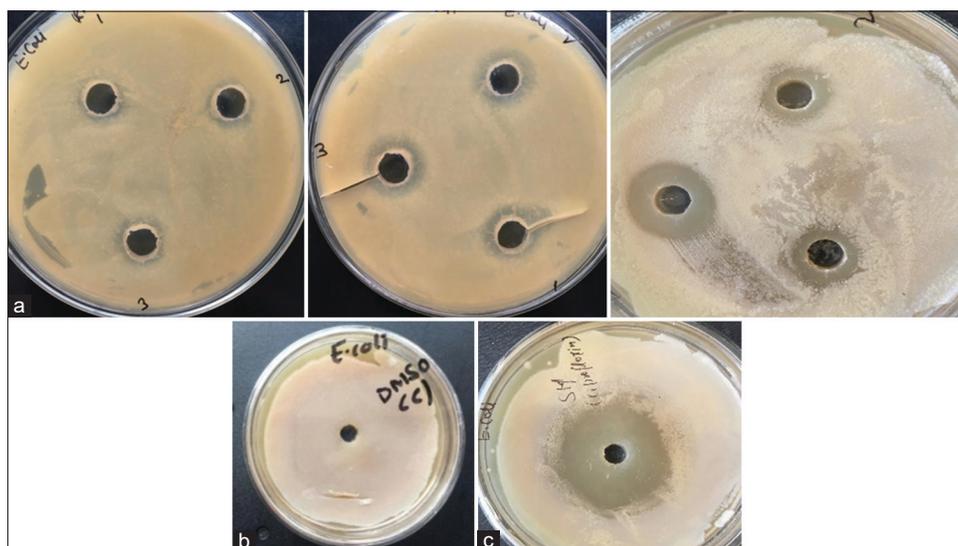


**Figure 5:** Job's plot of receptor with bicarbonate and carbonate at  $\lambda = 619$  nm

Job's plot was employed to determine stoichiometric ratios for both complexes at  $\lambda_{\text{max}} = 619$  nm. The inflection point was observed at 0.75 w.r.t. anion which implied the stoichiometry to be 1:3 for **1**, and 2:3 for **2** as shown in Figure 5.

## Antimicrobial activity

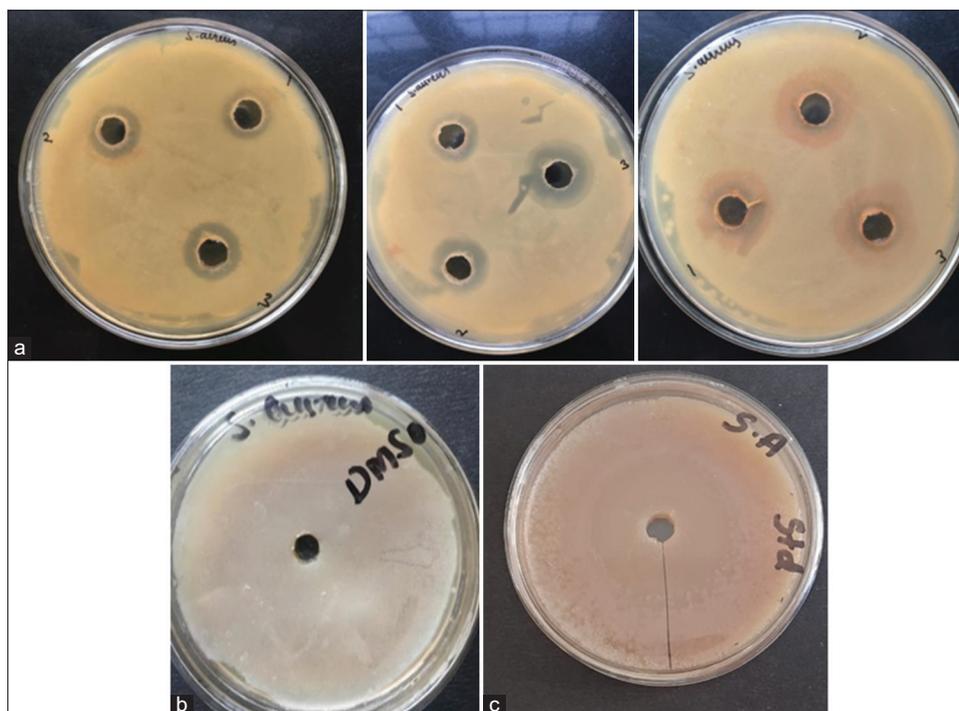
The anti-microbial effects were determined in different concentrations such as 50, 100 and 200  $\mu\text{g}/\text{mL}$ . The maximal inhibition zone value for *E. coli*, *S. aureus* and *A. niger* of the  $[\text{HuCr}]\text{Cl}_3$ , compounds **1** and **2** were recorded at 200  $\mu\text{g}/\text{mL}$  concentration. However, standard (ciprofloxacin and fluconazole) had a significant effect against all these



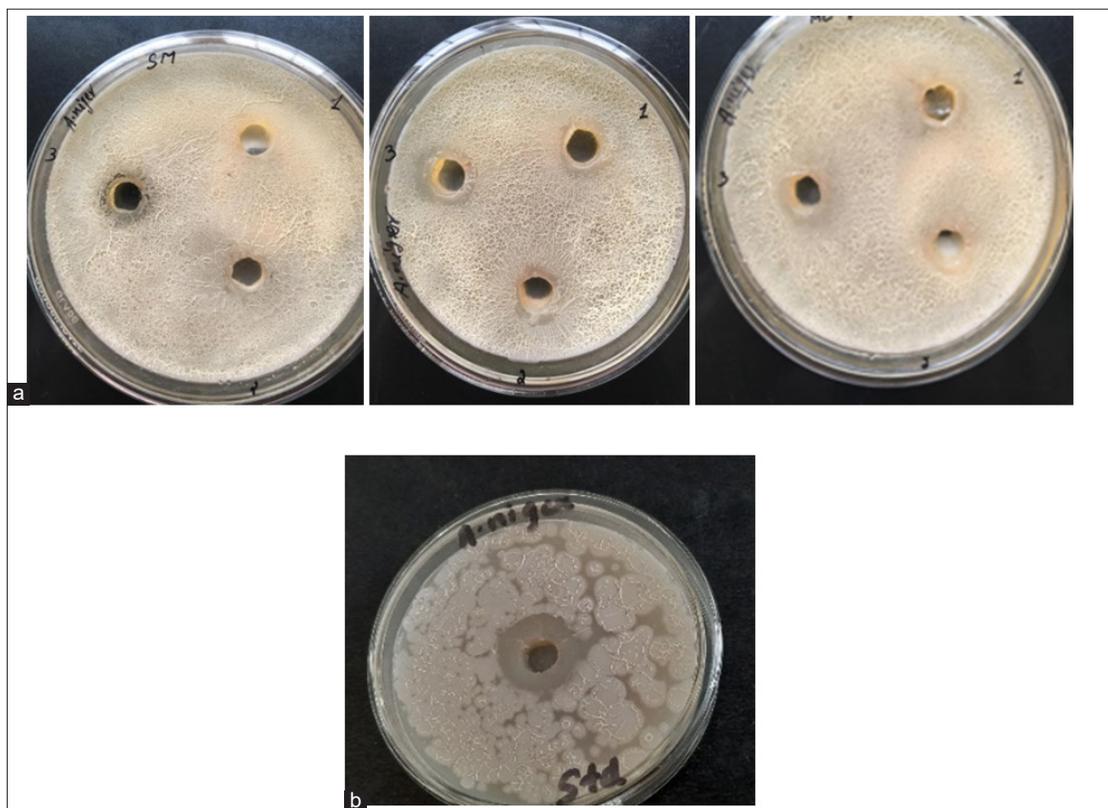
**Figure 6:** (a) Effect of antibacterial activity of  $[\text{HuCr}]\text{Cl}_3$ , **1** and **2**, (b) with Control (dimethylsulfoxide), (c) with standard (ciprofloxacin) against *Escherichia coli*

microbial strains. The compounds **1** and **2** were found to be very potent against the tested organisms as shown in

Figures 6-8. The antimicrobial activity was determined by measuring the inhibition zone in millimeters around the well



**Figure 7:** (a) Effect of antibacterial activity of  $[\text{HuCr}]\text{Cl}_3$ , 1 and 2, (b) with Control (dimethylsulfoxide), (c) with standard (ciprofloxacin) against *S. aureus*



**Figure 8:** (a) Effect of antifungal activity of  $[\text{HuCr}]\text{Cl}_3$ , 1 and 2, (b) with standard (fluconazole) against *Aspergillus niger*

**Table 2:** Log  $\beta$  of newly synthesized  $[\text{HuCr}]^{3+}$  complexes

Complex cation $[\text{HuCr}]^{3+}$ with anion	log $\beta$
$\text{HCO}_3^-$	10.534±0.0175
$\text{CO}_3^{2-}$	15.7377±0.0064

**Table 3:** Antimicrobial activity of  $[\text{HuCr}]\text{Cl}_3$ , compounds 1 and 2

Compounds	Doses ( $\mu\text{g}/\text{mL}$ )	Well-diffusion method (inhibition zone, diameter (mm))		
		<i>E. coli</i>	<i>S. aureus</i>	<i>A. niger</i>
$[\text{HuCr}]\text{Cl}_3$	50	0.5±0.2	1±0.2	-
	100	1±0.1	2±0.1	-
	200	2±0.1	3.5±0.2	1±0.1
Compound 1	50	1±0.12	1.1±0.1	-
	100	2.2±0.1	2.6±0.1	-
	200	4.2±0.1	5±0.2	0.5±0.1
Compound 2	50	1.2±0.2	1.5±0.1	0.5±0.3
	100	2.5±0.1	3±0.1	1±0.2
	200	3±0.1	5.7±0.2	2±0.1
Ciprofloxacin	100	20.5±0.1	20±0.1	-
Fluconazole	100	-	-	18±0.1

*E. coli*: *Escherichia coli*, *A. niger*: *Aspergillus niger*

and compared with ciprofloxacin and fluconazole presented in Table 3.

## CONCLUSION

The ionic reactions between anion receptor  $[\text{HuCr}]^{3+}$  and carbonated anions ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) in aqueous solution has been investigated and characterized by spectroscopic (IR and UV-visible) techniques. The binding properties of this cation with carbonated anions ( and ) in aqueous medium have been determined with standard UV-visible spectrophotometer through titration method (log  $\beta$  for  $\text{HCO}_3^-$  = 10.534,  $\text{CO}_3^{2-}$  = 15.7377). The results obtained indicate that the binding ability of the cation  $[\text{HuCr}]^{3+}$  towards carbonated anions is in the order  $\text{CO}_3^{2-} > \text{HCO}_3^-$ . Thus the selectivity of receptors for particular anion can be chosen. The results predicts that the octahedral geometry of  $[\text{HuCr}]^{3+}$  is capable enough to target both the carbonated anions.

All compounds were found to be potent against the tested organisms (*E. coli*, *S. aureus* and *A. niger*) and it was found that  $[\text{HuCr}]\text{Cl}_3$ , 1 and 2 shows maximum zone of inhibition at 200  $\mu\text{g}/\text{mL}$  concentration but the starting material i.e.  $[\text{HuCr}]\text{Cl}_3$  and compound 1 does not show any activity against *A. niger* at 50 and 100  $\mu\text{g}/\text{mL}$  concentration.

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