Evaluation of antioxidant properties of some gemcitabine-metal complexes

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Abstract. Some new gemcitabine complexes with some metal (II) chlorides (MCl₂) have been prepared in 2:1 molal ratio (Gem : MCl₂), where M= Cu(II), Co(II). The prepared complexes have been characterized by FTIR, CHN, and 1H NMR spectroscopy. Antioxidant properties for these complexes were evaluated and they showed lower antioxidant properties than the gemcitabine (Gem) alone.

1. Introduction
Gemcitabine is known drug for treatment of some types of cancers. Its trade name is gemzar, and it is nucleoside pyrimidine. Gemcitabine IUPAC name is 4-Amino-1-(2-deoxy-2, 2-difluoro-β-D-erythropentofuranosyl) pyrimidin-2(1H)-on. It is used for treatment of pancreatic cancer, non-small cell lung cancer, ovarian cancer, breast cancer and bladder cancer [1, 2]. Some derivatives have been prepared [3-5]. It has two hydroxyl and one amine groups in its structure, and thus it can coordinate to many metal ions to give complex compounds. The cytotoxicity effect of disulfiram glioblastoma cell lines and ALDH-positive cancer –stem-like cells have been reported, and showed that the effect of DS and combination of DS and gemcitabine (dfdc) on GBM CELLS stem-kike cells was investigated [6]. A mixture of rp\sp gemcitabine-[phenyl-benzyloxy-1-alaminyl] phosphate in the presence of catalyst comprising a metal salts selected from the group consisting of salts of Cu, Fe, La, and Yb [7]. In the present article we have prepared two metal complexes of gemcitabine by reacting gemcitabine with Cu(II), and Co(II) ions, in (2: 1) molal ratio and studying their antioxidant properties.

2. Experiment
2.1. Materials and apparatus
Gemcitabine drug was purchased from Max Hospital, New Delhi, India. Metal halides were from Murck (99.9%). Solvents were from BDH and used without further purification. Elemental analysis were performed on VarioEL CHNS. FTIR spectra were recorded using Shimadzu FTIR- 8400 spectrophotometer by using KBr disc, in the range (4000- 400) cm\(^{-1}\). H NMR spectra were recorded on Bruker Ultra shield 400 MHz using DMSO as a solvent and TMS as internal standard.
2.2. Method
2.2.1. General method:

The two gemcitabine metal complexes (1) & (2), were prepared by adding gradually 5 mmol of MCl₂, where M= Co²⁺ or Cu²⁺, dissolved in absolute ethanol, to 10 mmol gemcitabine (Gem), dissolved in absolute ethanol and stirred for about 30 to 60 min., at (25- 60) °C. Different color precipitates with different yields were formed. Then each complex was washed with distilled water, dried and kept in desiccator. Some physical properties are given in Table (1).

2.2.2. Preparation of [ Cu (Gem)₂ ] complex (1)

Gemcitabine (10 mmol); (2.63198 gm) was dissolved in absolute ethanol and made basic with 20 mmol of Na OH solution. Then 5 mmol of Cu Cl₂ (0.67223 gm, dissolved in absolute ethanol), was added gradually to the above solution and stirred for 30 min at 25 °C to give blue color precipitate, then was worked out as above item (2.2.1) to give blue precipitate (70% yield), and kept in desiccator.

2.2.3. Preparation of [ Co (Gem)₂ ] complex (1)

Gemcitabine (10 mmol), (2.63198 gm) was dissolved in absolute ethanol, and made basic NaOH solution. Then 5 mmol, (0.6492 gm) of Co Cl₂, dissolved in absolute ethanol, was added gradually to the above solution to give dark brown color precipitate (68 % yield), and then kept in desiccator.

Table (1) Some physical properties of Gemcitabine-metal complexes (1) & (2)

| Metal ion | Ligand | Reaction Temp. °C | Complex formula | color       | M.P. °C   |
|-----------|--------|-------------------|-----------------|-------------|-----------|
| Cu²⁺      | L1     | 25                | Cu(L1)₂        | Blue        | 260 dec.  |
| Co²⁺      | L1     | 60                | Co(L1)₂        | Dark Brown  | 281 dec.  |

2.3 Antioxidant properties:

Linoleic acid (0.02) ml was mixed with (Tween 20) in (100 ml) round bottom flask, then 1 ml of B- Carotene, (prepared chloroform), was added to the mixture above. Then chloroform was evaporated at room temperature and at the dark, and 50 ml of deionized water was added to the mixture. The resultant solution was divided into 5 ml portions and put in test tubes and each one containing 0.2 mg of the two complexes (1) or (2), and the third one containing BHT dissolved in 0.2 ml DMSO. Then the control solution was prepared by mixing 5 ml of Solution containing 0.2 ml DMSO. UV – visible absorption was measured directly at 470 nm for all samples. All test tubes were heated in water bath at 45 °C. The molar absorption for each sample were measured at 470 nm after each 15 min. and within a period (15 -105) min. And the absorbance of BHT solution were measured at 470 nm too with time. Then the antioxidant activity of the two complexes (1) and (2) were compared with BHT activity as shown in fig (3) which indicates the relation between absorbance and time at fixed (λ) according to the following equation:

\[ AA = \{ 1 - (A_j - A_{j\cdot} - A_{t\cdot}) \} \times 100, \]

where

- \( AA \) = % activity of sample as antioxidant reagent.
- \( A_j \) = Absorbance of sample at (0) time.
- \( A_{1\cdot} \) = Absorbance of sample at 105 min.
- \( A_{j\cdot} \) = Absorbance of control solution at (0) time.
- \( A_{t\cdot} \) = Absorbance of control solution at 105 min.

3. Result and Discussion:
3.1. Elemental analysis:
Elemental analysis data of the complexes (1) and (2) shows that the coordination is no. 4 and the ratio of (metal: Gem) is (1:2) as given in Table (2).

Table 2: Elemental analysis data.

| Complex compound | % C (P) / (Theory) | % H (P) / (Theory) | % N (P) / (Theory) |
|------------------|--------------------|--------------------|--------------------|
| [Cu(Gem)₂]       | 36.9984 / 36.6469  | 4.2612 / 3.7559    | 14.8359 (P) / 14.2456 |
| [Co(Gem)₂]       | 37.5410 / 36.9358  | 3.9736 / 3.7886    | 14.8503 (P) / 14.3579  |

3.2. FTIR spectra:
FTIR spectrum of [Cu (Gem)₂] complex (1) shows nearly similar bands of gemcitabine but some deviations of certain bands occur. Two amine-bending vibrations bands are shifted to lower frequencies occurred at 1663 cm⁻¹ and a weak band embedded as a shoulder at about 1625 cm⁻¹ in contrast to 1674 cm⁻¹ and 1662 cm⁻¹ in gemcitabine ligand. This is may be due to coordination of Cu(II) ion to nitrogen of amine group, whereas another stretching band occurs at 3388 cm⁻¹ in contrast to gemcitabine ligand occurred at 3386 cm⁻¹ which is may be due to the stretching of NH₂ protons are not affected by coordination as that of bending vibration. Stretching band for C=O occurs at about 1694 cm⁻¹ for the complex and in the same stretching frequency for the ligand, which indicates that Cu²⁺ does not coordinate to carbonate oxygen atom. For Co²⁺ complex [Co (Gem)₂] (2), gave nearly similar bands as that of the ligand, except that of the to bending bands of (NH₂) group are shifted to lower frequencies ; 1644.98 cm⁻¹ and the other band at about 1550 cm⁻¹, which is may be due to the coordination to Co²⁺ ion. And ν_sym of C=O is in the same stretching frequency as of the ligand therefore it indicates that Co²⁺ does not coordinate to oxygen of carbonyl.

3.3. ¹H NMR spectra:
The proton NMR spectra of the two complexes (1) and (2) show similar bands as that of gemcitabine ligand (Gem), but with little shift of some bands to lower field due to coordination to the metal ions. For first complex, i.e. [Cu(Gem)₂] (1), CH₂ band (-CH₂-OH) occur at 3.64 ppm, whereas for (Gem) ligand occurs at 3.5 ppm and mixed with DMSO band occurs at 3.5 ppm as well that increase the integration value to 20.04. And for the two (OH) groups, one which is attached directly to the (5-ring) group occurs at 4.15 ppm and the ligand occurs at 4.37 ppm, and second occurs at 3.65 ppm, compared to (Gem) occurred at 3.94 ppm. The greater difference in chemical shift is for NH₂ group- protons that occur at 6.28 ppm, whereas it occurs theoretically at 6.68 ppm. This difference in NH₂ chemical shift compared to another atoms chemical shifts is may be to the coordination of Cu²⁺ ion to nitrogen atom of amine group. The second coordination bond may be with either N atom in the heteroatom ring, or with oxygen of carbonyl at C no. 8. But the probability of coordination to O atom of carbonyl is more than that of N atom in heteroatom giving (Ring=6) complex with Cu²⁺ ion, which is more stable than if the coordination will be with O atom of carbonyl due to the presence of two double bonds with the ring. For ¹H NMR spectra of [Co(Gem)₂] complex (2) show similar band as the ligand (Gem) but with little shifts due to coordination with Co²⁺. And as with the Cu²⁺- complex, the larger chemical shift is for NH₂ group that occurs at about 6.24 ppm compared to free gemcitabine, occurs at 6.28 ppm. Therefore Co²⁺ may coordinate as for Cu²⁺ complex (1) via (N) atom of (NH₂) group and (O) atom of C=O at C no.8, and for the same reason as in the Cu²⁺-complex for attaining the stability in (Ring=6).
The details of $^1$H NMR data is shown in Table (3) and in Figs (1) and (2). Co$^{2+}$ is in the borderline, as the case of Cu$^{2+}$, in regard to (acid/base) hardness too. Some extra bands may be due to some excipients with gemcitabine drug.

Table (3) $^1$HNMR Data

| Complex Cpd. | (OH)-CH | (OH)-CH | NH$_2$ | CH-a | CH-b | CH-c | CH-d | CH-e |
|--------------|---------|---------|--------|------|------|------|------|------|
| [Cu(Gem)$_2$] | 4.15    | 3.65    | 6.28   | 6.3  | 8.05 | 4.23 | 4.15 | 3.87 |
| [Co(Gem)$_2$] | 4.19    | 3.75    | 6.27   | 6.05 | 7.67 | 4.1  | 4.2  | 3.75 |

Figure 1. Structure of gemcitabine (Gem.)

Figure 2. H NMR spectra of [ Cu (Gem)$_2$](1).
Figure 3. H NMR spectra of [Co(Gem)$_2$](2).

The suggested structure for both complexes are square planar or tetrahedral complex for [(Cu(Gem)$_2$) and [Co(Gem)$_2$]. And for prediction of both structures, one should do single crystal spectra, if possible in the future.

3.4. Antioxidation properties

The mechanism of reduction reaction for B–Carotene is accompanied by oxidation of Lionleic acid and forming linoleic hydroperoxide, which attack the unsaturated B–carotene molecules. And when B–carotene molecule double bods lose their double bonds by oxidation process, then the molecule loses its orange color. But if there are different antioxidant materials are present that can prevent reduction of B–carotene by scavenging linoleic radicals and any other radicals that are formed in the system, therefore the absorption will be reduced rapidly for samples which are free from antioxidant compounds, but with existence of any oxidant compounds, their color and absorption will last for longer time Ref.(70, 71, 72). The results of antioxidant properties for the complexes [Cu(Gem)$_2$] and [Co(Gem)$_2$] and the ligand (Gem) are listed in Table (3). And as shown in Figure 3, $A_t$ is greater for [Co(Gem)$_2$] than that of [(Cu(Gem)) , 0.36 and 0.345 respectively, therefore Co$^{2+}$–complex has little more antioxidant activity than Cu$^{2+}$–complex. But the toxicity property of the complexes have not been done, and if it will show that both complexes are less toxic than gemcitabine, then these complexes may be preferred as anticancer agents more than gemcitabine, if will be done in future.
Table (4) Data for Relation of absorbance of the complexes (1) & (2) and BHT with time (min).

| Time  | Control solution | Ligand | Co\(^{2+}\) complex (2) | Cu\(^{2+}\) complex (1) | BHT |
|-------|------------------|--------|--------------------------|-------------------------|-----|
| 0 min | 0.238            | 0.282  | 0.443                    | 0.43                    | 0.75|
| 15 min| 0.22             | 0.272  | 0.421                    | 0.412                   | 0.734|
| 30 min| 0.205            | 0.261  | 0.412                    | 0.395                   | 0.722|
| 45 min| 0.184            | 0.256  | 0.402                    | 0.379                   | 0.715|
| 60 min| 0.16             | 0.242  | 0.396                    | 0.361                   | 0.705|
| 75 min| 0.141            | 0.217  | 0.381                    | 0.35                    | 0.69 |
| 90 min| 0.122            | 0.205  | 0.368                    | 0.345                   | 0.685|
| 105 min| 0.111           | 0.205  | 0.36                     | 0.345                   | 0.685|

Figure 4. Relation of absorbance of the complexes (1) & (2) and BHT with time (min.)

Acknowledgment

We are gratitude for College of science, Basrah University for supporting the work. Our thanks for Isfahan University, Islamic Republic of Iran, for doing physical measurements.

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