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Anti-cancer study of Ni (Π) and Zn (Π)- chitosan complexes

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ABSTRACT

Two neutral chitosan polymer complexes with Ni(Π) and Zn (Π) were prepared and characterized by molar conductivity measurements, IR spectroscopy. The prepared complexes were tested against two types of cancer cell lines {breast cancer (MCF-7) and ovarian cancer (A2780)}. That the Ni-chitosan complex was significantly active then the Zn-chitosan complex with IC_{50} value is 167.67± 4.6 µg/ml for (MCF-7) and 200.45± 8.23µg/ml for (A2780). Whereas IC_{50} value of Zn-chitosan complex 189.03± 3.7µg/ml for (MCF-7) and 231.70± 5.25µg/ml for (A2780)

1. Introduction

Some terms must be considered while preparing or producing polymers as drug distribution or drug carriers systems such as molecular weight, bio-suitability, drug affinity, and drug release capability.

The polymeric materials degrade inside the living organism in process calls Biodegradation which is induced by biochemical factors such as enzymes or microorganisms such as bacteria. So polymers may classified depending on their biodegradability into: Non-biodegradable polymers or biodegradable polymers.[1,2]. Drug polymers may be used to decrease the toxicity and increase the selectivity for anti-cancer agents[3] by delivering drugs to specific locations or targets to cure many diseases.

The natural polymer chitosan (which is produced from marine animals' shells) is one of these polymers, and it's one of the most popular polysaccharides[4] with a high molecular weight. The chitosan ability of complexes` formation is due to the presence of the non-asymmetric electron pair on the nitrogen atom of the amine group[5]. While new safe and effective anti-tumor agents have been discovered and developed, chitosan in its composition can help to the administration of an active anti-cancer derivative, by interaction with its amino, hydroxyl, and acetamide groups, which can selectively permeate through cancer cell membranes.[6].

Cis-platin complexes have been used as an anti-tumor medicine since (50) years ago, but their uses were reduced now as their limited activity, as well as many side effects involved according to the dose given to treat the cancerous tumor[7]. As mentioned chitosan contains many groups such as hydroxyl, amine and acetamide thus due to its ability as a multi-dentate ligand[8-9] it coordinates with many metals ions such as platinum with copper, zinc and ruthenium.[10] In this study, we describe preparation two chitosan polymer complexes with Ni(Π) and Zn(Π), and tested the anti-cancer activity against ovarian and breast cancer cell lines.

2. Experimental Parts

2.1 Instrumentation

The FT-IR spectra have recorded using Shimadzu 8400 spectrophotometer (400 – 4000 cm^{-1}) with KBr disk, conductivity measurement were measured using 2500CD conductor meter at (10^{-3}M) of 2% acetic acid. The anti-cancer activity was tested against two types of cell line (ovarian and breast) in Al-Raziy Lab. Tahran university –Ira.

2.2 Synthesis of complex Zn- Chitosan (B4)

The Zn-chitosan complex was prepared according to the method describe in[11].

Chitosan polymer (0.500g, 3.100mmol) was added to a solution of $NiCl_2.H_2O$(0.295g: 1.24mmol) in 2% acetic acid (50ml) with stirred and neutralized with dilute ammonia solution till pH=7.1. The mixture was stirred for 3h , then cooled at room temperature. A green ppt. formed was filtered off, wished with absolute ethanol and dried under vacuum at °80C.

(**B4**) Green solid, yield 98%, m.p: 216 °C, molar conductivity in 2% acetic acid (10^{-3} M) at 25°C: 5.00

$\Omega^{-1}.Cm^{-1}.mol^{-1}$, IR(KBr) ($cm^{-1}$):3431, 3346, 1406, 1018, 451.

2.3 Synthesis of complex (B5)

Chitosan polymer (0.500g :3.10mmol) was added to a solution of $ZnCl_2$ (0.163 g: 1.20 mmol) in 2% acetic acid (50ml) with stirred and neutralized with dilute ammonia solution till pH=7.1, the mixture was stirred for 3h , then cooled at room temperature. A light brown ppt. formed was filtered off, wished with absolute ethanol[11] and dried under vacuum at 80C°.

(**B5**) Light brown solid, yield 99%, m.p:223 °C, molar conductivity in 2% acetic acid (10^{-3} M) at 25°C: 2.00 $\Omega^{-1}.cm^{-1}.mol^{-1}$, IR(KBr) ($cm^{-1}$):3433, 3317, 1404, 1016, 420.

3. Results and discussion

3.1 Synthesis

Treated two moles of neutral chitosan polymer with nickel chloride or zinc chloride in 2% acetic acid afforded chitosan polymer complexes (Figure 1). The prepared complexes have characterized by melting point, IR, and molar conductivity. The low molar conductivity indicates that the Ni-chitosan and Zn-chitosan complexes are non-electrolyte[12].

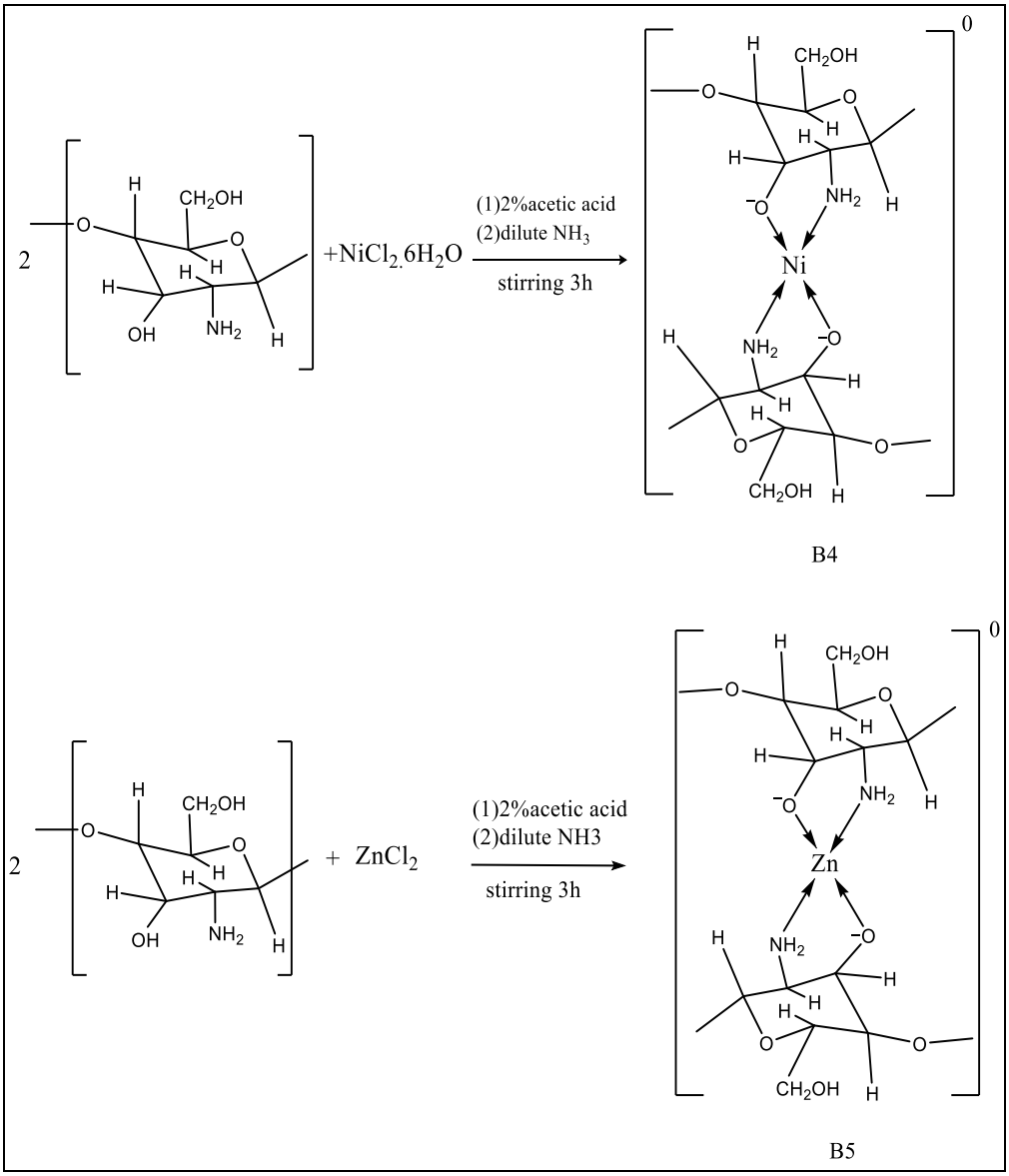

Fig. 1: preparation of chitosan complexes

3.2 FT-IR Spectral data

The infrared spectrum of the chitosan showed peaks at 3462, 3354, 1423, and 1157 cm^{-1} due to νO-H, νNH_2, νC-N, and νC-O stretching frequencies respectively, On the other hand, the infrared spectra of the prepared complexes B4, B5 showed shifts to lower frequencies for some of the peaks compared to the free polymer (as shown in table 1), these results support that the chitosan coordinates with metals at these groups, as well as νM-L stretching peaks appeared at 451 and 420 cm^{-1} for B4 and B5 respectively [13, 14].

Table1: FT-IR spectra data of the chitosan complexes(in cm^{-1}).

Comp.	ν(O-H)	ν(NH_2)	ν(C-N)	ν(C-O)	ν (M-L)
Chitosan	3462	3354	1423	1157	-------
B4	3431	3346	1406	1018	451
B5	3433	3317	1404	1016	420

3.3 Anti-cancer activity study

Many studies estimate the efficacy of chitosan (or its complexes) as an anti-cancer with good results [14-19], thus the effectiveness of two Ni-chitosan and Zn-chitosan complexes, against two types of cancer cells [breast cancer cell line (MCF-7) and ovarian cancer cell line (A2780)] have studied, using different concentration ranged between (5-500 µg), the cytotoxicity effect has evaluated then the rate of inhibition of cell growth has calculated and compared with cis – Platin (as standard drug) , Table 2 and Figures 2 and 3 showed the results obtained.

Table 2: (IC_{50}) values of the prepared compounds compared with Cis- platin.

Compounds	IC_{50} [a] of MCF-7 ± SD		IC_{50} [b] of A2780 ± SD	
	µg/ml	µM	µg/ml	µM
Zn-chitosan	189.03± 3.7	0.45± 0.04	231.70± 5.25	0.61± 0.10
Ni-chitosan	167.67± 4.6	0.36± 0.08	200.45± 8.23	0.52± 0.09
Cis- platin [c]	78.56± 1.23	0.17± 0.01	69.13± 0.98	0.19± 0.04

a. The (IC_{50}) half-inhibition concentrations of cancer cells were calculated after (24 hours) with different concentrations three times, depending on the (MTT) method.

b. (IC_{50}) calculated in (µM) units of the dose-response curve.

c. (cis-platin) used as a standard reference.

The antitumor activity was decreased with the decreasing of concentration (5µg), whereas the activity was increased at the highest concentration (500µg). The percent of viability cells of breast cancer which treated with nickel complex is (27.78%) while for zinc complex is (36.19%), furthermore, the percent of viability cells of ovarian cancer which treated with nickel complex is (23.61%), while for zinc complex is (34.91%).

The IC_{50} value (which represents half inhibition concentration of cancer cells growth) at MCF-7 for Ni-chitosan is 167.67± 4.6µg/ml, while for Zn-chitosan is 189.03± 3.7µg/ml. on the other hand, at A2780 the IC_{50} value for Ni-chitosan is 200.45± 8.23µg/ml, while for Zn-chitosan is 231.70± 5.25µg/ml [23-25].

The results show that Ni-chitosan is more effective than Zn-chitosan, which agrees with previous research that copper, nickel, and some transitional elements complexes have more activity than the zinc complexes.[15-22].

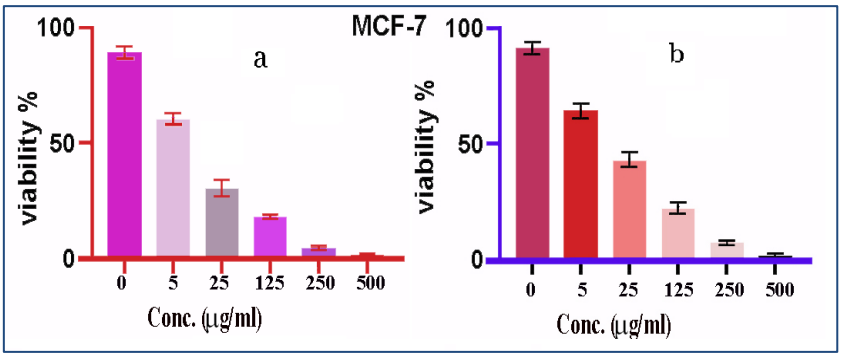

Fig. 2: Inhibitory activity of the complexes aganist breast cancer cells growth (MCF-7): (a)Zn-chitosan. (b) Ni-chitosan

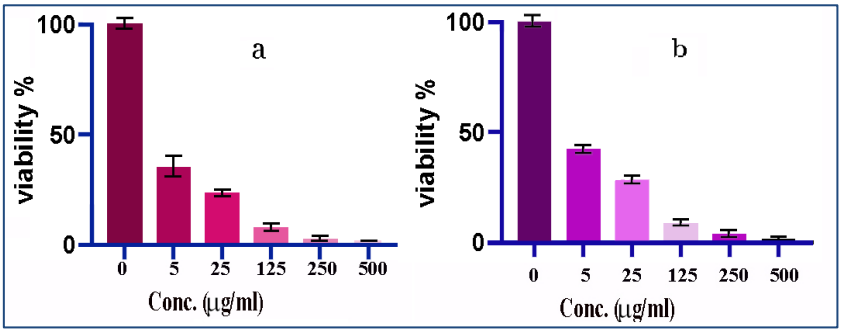

Fig. 3: Inhibitory activity of the complexes towards ovarian cancer cells growth (A2780): (a) Zn-chitosan (b) Ni-chitosan

4. Conclusion

The activity of the prepared complexes on the ovarian cancer cells growth (A2780) was higher than in breast cancer (MCF-7), as well as there is a direct relationship between the inhibition effect of the prepared compounds and its concentrations, moreover Chitosan-Ni was more active as an anti-cancer than the Chitosan-Zn.

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تحضير ودراسة فعالية معقدات الكيتوسان كمضاد لبعض انواع السرطان

بيادر رسول عبد ، علي اعميري محمد

قسم الكيمياء ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

في هذا البحث تم تحضير معقدات من البوليمر الطبيعي الكيتوسان مع النيكل (II) والخارصين(II), وشخصت بواسطة قياس التوصيلية المولارية وطيف الاشعة تحت الحمراء. تم اختبار المعقد المحضر مع نوعين من الخلايا السرطانية, سرطان الثدي (MCF-7) وسرطان المبيض (A2780) . وقد وجد ان المعقد (Ni-chitosan) اكثر فعالية من معقد (Zn-chitosan) وان قيمة IC_{50} لمعقد(Ni-chitosan) (167.67± 4.6 µg/ml) لسرطان الثدي و (200.45± 8.23µg/ml) لسرطان المبيض. بينما قيمة IC_{50} لمعقد (Zn-chitosan) 189.03± 3.7µg/ml لسرطان الثدي و 231.70± 5.25µg/ml لسرطان المبيض.