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Research Article

Synthesis and Characterization of Novel Nano Graft Copolymer-N-acetylpseudoephedrine drug Composite and Studying their Effect in the Treatment of Lung Cancer

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Abstract

The research includes two steps: in the first step, preparation of a nano co-polymer from the reaction of one mole of glycerol with three moles of phthalic anhydride and was chracterized using ^{1}H-NMR, FT-IR, TEM, AFM. The second step is the loading of the drug (N-acetyl pseudoephedrine) onto the nano co-polymer via ester bonds. In order to determine the protein binding site for the amino acid bond under investigation, an AutoDock software was used to investigate molecular docking. The above-mentioned chemotherapy drug was tested on a lung cancer cell line, and the results showed its high effectiveness in preventing the spread of lung cancer cells, as evidenced by the noticeable decrease in the percentage of these cells compared to the control group. Novel cytotoxicity of nano composites containing N-acetyl pseudoephedrine in A549 cells. The IC50 value is 45.033 µg/mL.

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1. Introduction

Lung cancer is one of the main causes of death in a lot of nations worldwide. [1-4] Due to the rapid rise of this disease, scientists are looking for novel remedies. Among the most popular cancer treatment methods include radiotherapy, chemotherapy, and surgical excision. [5] medicine resistance, which indicates that these therapies have no negative side effects, is one of the many treatment-related issues that develop when a medicine is not able to reach the tumors completely at a sufficient dosage. [6] Because tumors are not provided enough concentration during these treatments, a variety of issues arise, including the establishment of drug resistance in cancer patients, making the negative consequences of these treatments poorly concealed. Consequently, in order to combat lung cancer, scientists need to employ novel and accurate techniques. [7, 8]

This is the reason behind the development of nanotechnology, which uses particles between 1 and 100 nanometers in size. These molecules can bind to any chemical substance, including anti-cancer drugs, antibodies, and other substances. [8, 9] As a result of their minuscule size (100-1000) times smaller than cancer cells nanoparticles can readily enter blood arteries and engage in interactions with both internal and external proteins. [10, 11] As a result, these molecules serve as a precise carrier that delivers the medication to the cancer cell, and researchers consider this to be a novel therapeutic weapon against cancer cells. [12,13]

Many methods of delivering medications have been developed, some of which use nanoparticles like synthetic polymers. In an effort to increase the drug's biological efficacy and accumulation in the intended cell, this was done. This is important for anti-cancer drugs since it guarantees that the drug reaches the intended cell at the right dosage without damaging healthy tissue.The drug deposited on the nanoparticles gradually degrades, enabling the precise delivery of therapy to the sick cell. As a result, these factors have been crucial in the clinical adoption of nanotechnology-based drug delivery. [14]

2. Material and method

All necessary chemical analyzes were used

2.1 Synthesis of new nano graft co-polymer (Z1)

In a 125 mL beaker, we mixed 3.0 mol, 27.5 g of phthalic anhydride, with 7.5 mL of DMSO solvent. After that, the mixture was constantly stirred and carefully heated to 130°C until the solution turned into a transparent liquid. After that, added glycerin (1.0 mol, 5.75 g) to the mixture. We noticed bubbles resulting from the release of water, so we added 12 mL of xylene gradually until the bubbles disappeared. Then added cold deionized water to precipitate the resulting solution. The precipitate was then filtered and washed with additional deionized water, and the precipitate was left to dry at room temperature. Thus, the required polymer was obtained using condensation polymerization.

2.2 Synthesis of acid chloride for the nano graft co-polymer (Z2) [15]

In a 25 ml beaker, mixed (0.6 g) of compound Z1, five drops of thionyl chloride (SOCl2), and 6 mL of DCM solvent. After we mixed the above mixture using a magnetic stirrer, after 30 min we left the mixture at room temperature with constant stirring, at 65 °C. After two hours, the temperature was raised to 85°C and the acid chloride of the nano graft co-polymer (Z2) was prepared.

2.3 Synthesis of nano graft Co-Polymer-Drugs [16]

The drug loaded on the nano co-polymer was prepared by mixing the previously prepared

compound (Z2) with the drug (N-acetyl pseudoephedrine). At 85°C, (500 µL) Et3N and (5 mL) DCM were added to the mixture with continuous stirring. After that, the mixture was left at the same temperature without stirring until it dries and the desired compound was obtained.

2.4 Molecular Docking [17]

Many studies have shown the great importance of molecular docking, as it determines the type and properties of the prepared nano composites. In addition, it demonstrates the most efficient binding energy for drugs used to bind genes and proteins of lung cancer cells.

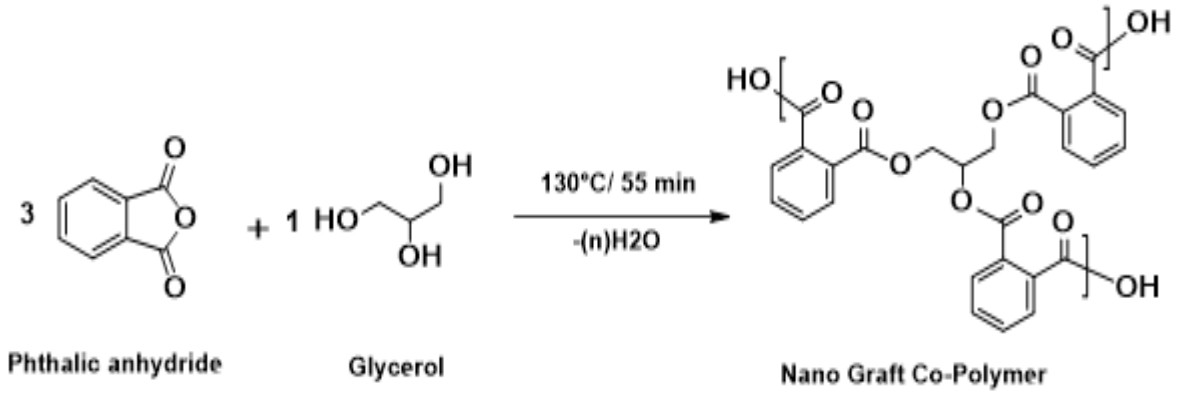
2.5 Cytotoxicity Assays [18]

The studies were conducted following the methodology stated in the references, and the MTT cell viability assay was carried out using 96-well plates to measure the cytotoxic effect (Subjects x) and the impact of the generated co-polymer drug graft complexes on lung cancer metastasis.

3.Result and discussion

3.1 Synthesis of new nano graft co-polymer (Z1)

The nanoparticle-grafted copolymer (Z1) was formed using a condensation polymerization process. This polymerization takes 55 mins between (1mol) of glycerol and (3 mol) of phthalic anhydride at 130°C. Water appears as a byproduct. As in **Scheme 1**. Then FT-IR, ^{1}H-NMR, AFM, DSC, TEM, and XRD were used to analyze this co-polymer.



Scheme (1): Preparation of copolymer for nanografting

Table (1) shows the absorption peaks of the FT-IR spectrum and as described in **Figure (1)**

(3075 cm^{-1})	(3005 cm^{-1})	(1669 cm^{-1})	(1069 cm^{-1})	(734 and 897 cm^{-1})
Weak broad bond(O-H) alcoholic and H-bond	stretching band bond (C-H) aromatic	strong stretching band Bond(C=O) ester	Bond (C-O) ester	Attributed to di substitution of (aromatic ring)

Figure 2: ^{1}H-NMR spectrum. Compound (Z1) appears as a double signal for (ph.-H) at 8 ppm and as a signal for acid (OH) at 13 ppm.

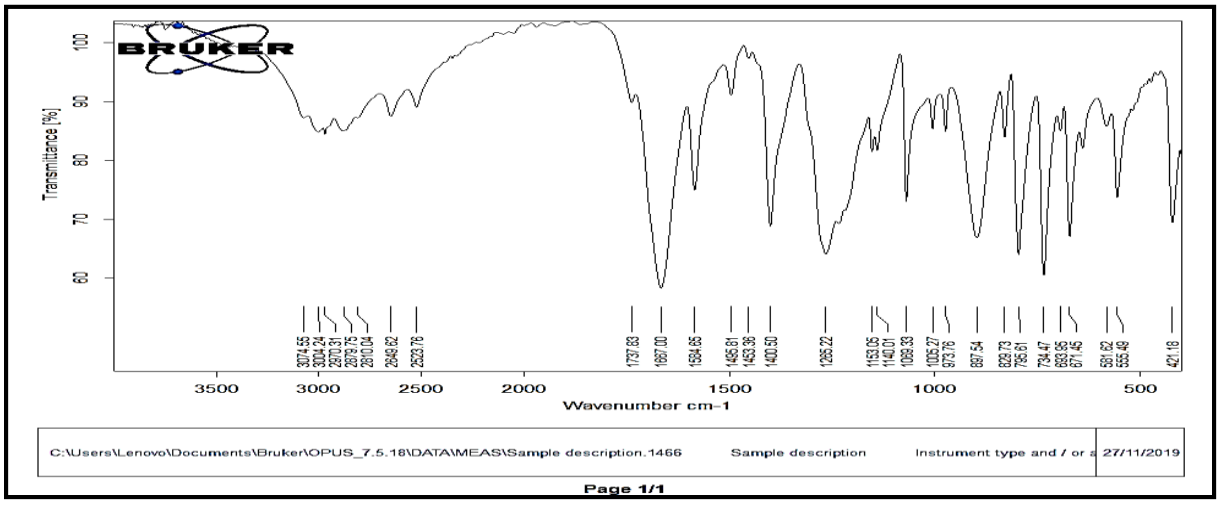


Figure (1): FT-IR spectra of the nanograft co-polymer compound (Z1)

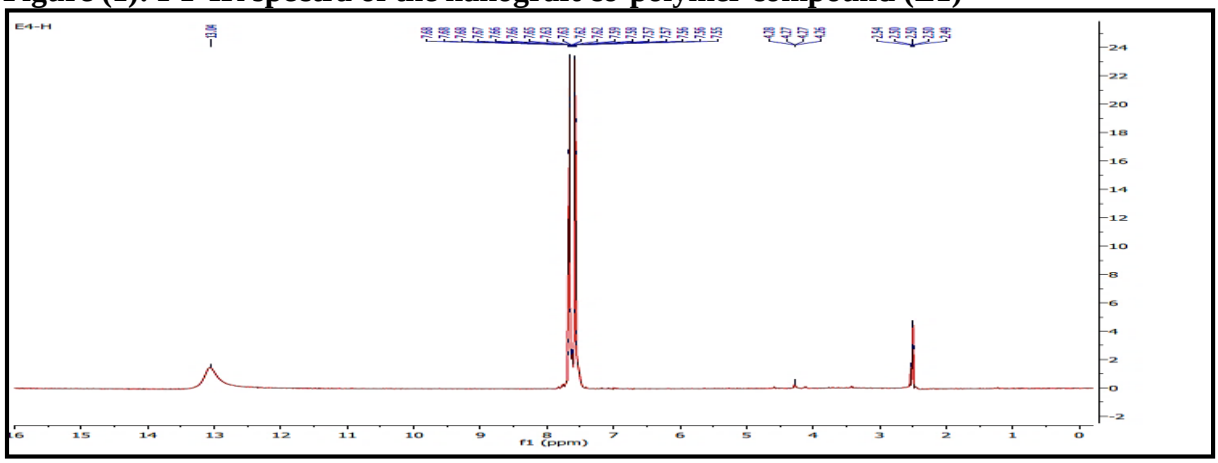


Figure (2): ^{1}HNMR spectrum of the nano graft co-polymer compound (Z1)

Figure 3 (a and b) of the (AFM) depicts the exterior of the co-polymer nanoparticles. The co-polymer surface's square root square was 5.94 nm, and its roughness coefficient was 5.08 nm. This suggests that the surface roughness, homogeneity, and homogeneous crystalline structure are all significantly influenced by the large size of the nanoparticles. Furthermore, as shown in **Figure 3a**, the average particle height was equivalent to 22.04 nm. The findings indicate that the co-polymer (Z1) nanoparticle's molecular size was 68.62 nm. **Table 2** displays the different ratios of these volumes along with the overall rate of the common nanoparticle particle sizes.

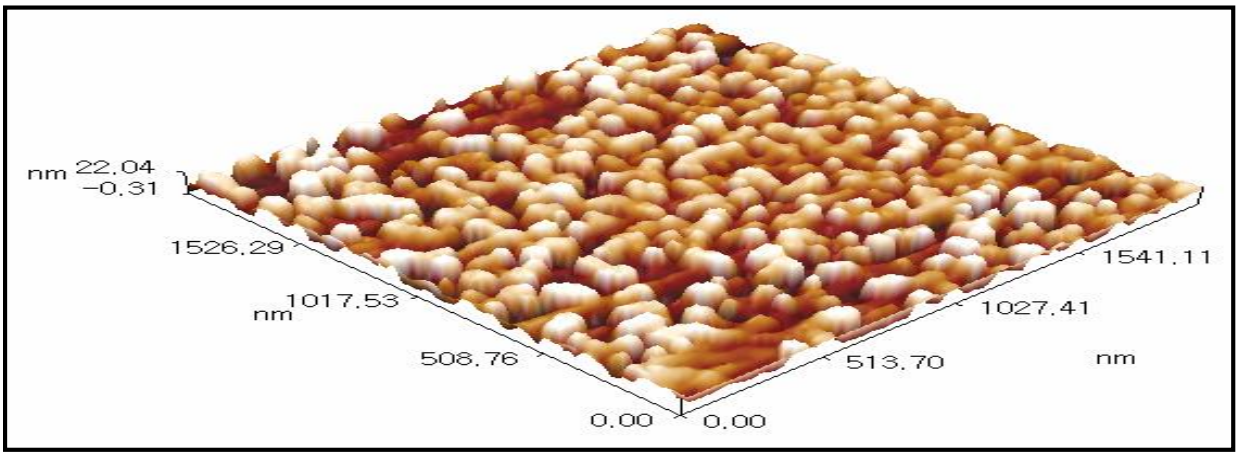


Figure (3a): The three-dimensional image of the nanograft co-polymer is shown using the Atomic Force Microscope.

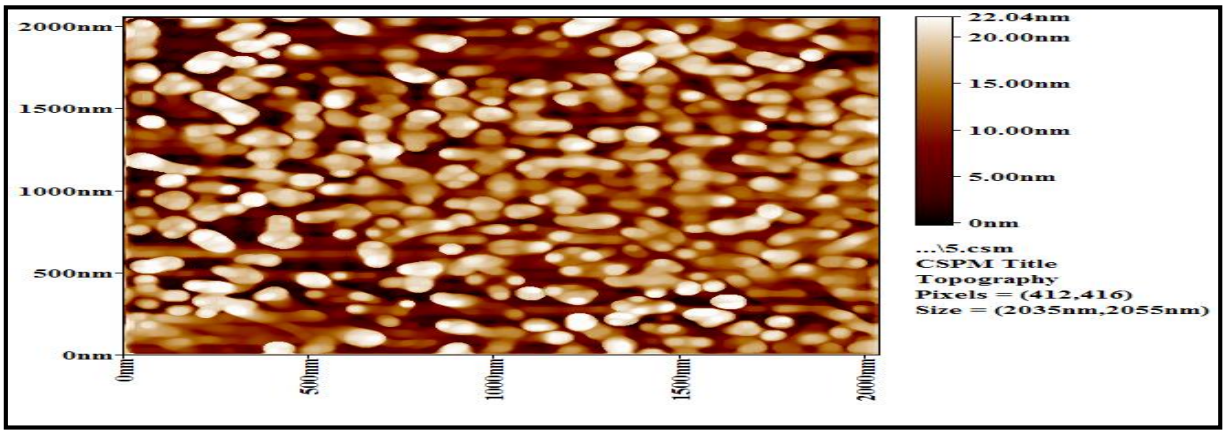


Figure 3b: A 2D atomic force microscope (AFM) image of the nano-grafted copolymer is shown.

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Table 2: The relative volumes of the different volumes as well as the overall rate of the nano co-polymer nanoparticle particle sizes

Sample: 1	**Code:** Sample Code
Line No.: lineno	**Grain No.:**1264
Instrument: CSPM	**Date:** 2023-11-24

Avg. Diameter: 68.62 nm	**<=10% Diameter: 50.00 nm**
<=50% Diameter: 65.00 nm	**<=90% Diameter: 80.00 nm**

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
45.00	1.52	1.52	65.00	17.80	40.91	85.00	11.74	90.91
50.00	4.17	5.68	70.00	12.12	53.03	90.00	9.09	100.00
55.00	9.09	14.77	75.00	13.64	66.67			
60.00	8.33	23.11	80.00	12.50	79.17			

A diffuse halo at 2q = 20, which is connected to the intra-chain segment distance, was visible in the nano polymer's x-ray diffraction (XRD) patterns, as seen in **Figure 5**. Each polymer sample's diffractogram contains more distinct peaks. The XRD pattern suggests that, in contrast to polymers that are entirely aliphatic, stiff aromatic rings produced from phthalic acid lead to more rigid structures, which should result in a larger potential for crystallization. More carbonyl groups were present in the polymer synthesis due to the phthalic acid to glycerol molar ratio utilized. In addition, higher phthalic acid concentrations implied that molecular motions brought on by the stiffness of aromatic rings would facilitate the ordering of polymer chains in crystalline lattices. **Figure 5**, demonstrates the use of Origin software to obtain X-ray diffraction (XRD) for the co-polymer of nanoparticles. Bragg's Law states that the average inters planer distance (dhkl) between atoms was 0.416 nm, and this value agrees with AFM values in above.

$n\lambda = 2d\sin\theta$ …… Bragg's Law

Scherrer's equation indicates that each crystallite's average size was 68.487 nm.

$D = \frac{k\lambda}{\beta\cos\theta} q$ …….. Scherrer's Equation

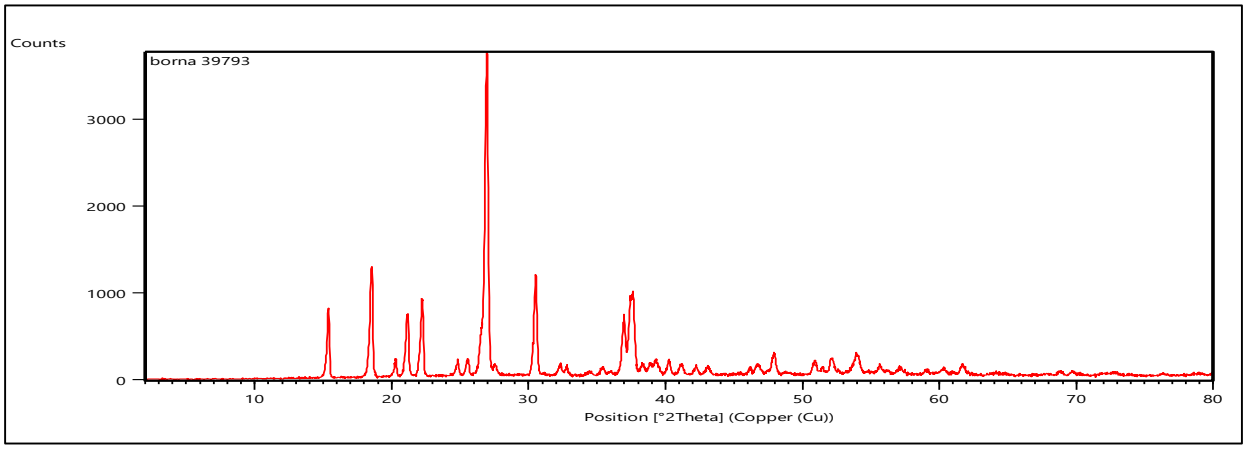


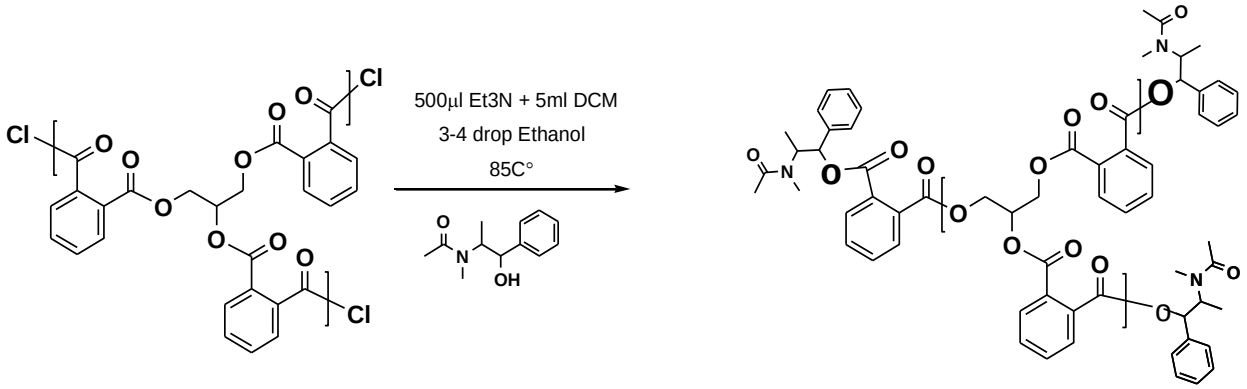
Figure 5: X-ray diffraction study of copolymer nanoparticles

Table 3 displays the ratios of the crystalline diameters of the copolymer to the atom-to-atom spacing (d-spacing).

2 θ	θ	FWHM	D nm	d_{hkl} nm	D (Av.) nm	d_{hkl} (Av.) nm
15.41888	7.70944	0.10308071	77.77159	0.57421	**68.4874**	**0.4152**
18.56158	9.28079	0.12176233	66.10961	0.477637		
21.20363	10.60181	0.11615246	69.58317	0.418681		
22.26382	11.13191	0.11743049	68.94823	0.398978		
26.9992	13.4996	0.13275784	61.54072	0.32998		
30.55913	15.27956	0.12296861	66.97155	0.292302		

3.2 Synthesis of drug nano graphite copolymer (Z3)

An amount (0.6 g) of compound (Z2) reacted with 500 µl Et3N and 5 ml DCM at a temperature of 85 °C after reacting with (0.19g) of the drug (N-acetyl pseudoephedrine) and 3-4 drops of ethanol to get rid of the remaining solvent. The equation represents the synthesis of the copolymer (z3).



Scheme (2): Composition of compound (Z3)

Figure (6) displays the FT-IR spectrum of compound Z3. Which appears as shown in the following **table (4)**.

(2972 cm^{-1})	(1724cm^{-1})	(1556 cm^{-1})	(1471 cm^{-1})	(1074 cm^{-1})	(1251.80cm-1)
C-C-Haliph	C=O ester	C=O amid	C=C ph	C-O	C-N

Figure (7), ^{1}H-NMR spectra are shown: signal at 3.1ppm for (C=C-H)ph, signal appears at 3.7 ppm for (NH-C=O)amid, signal appears at 2.5ppm for (C=O) ester intermediate; A signal appears at 2.9ppm for (C-H3).

^{13}C-NMR spectra **Figure (8)**: It appears at a signal of 169 ppm for the C=O ester, a signal of 155ppm for (NH-C=O)amid, and a signal of 135ppm for $(H\text{-}C{=}C)_{PH}$.

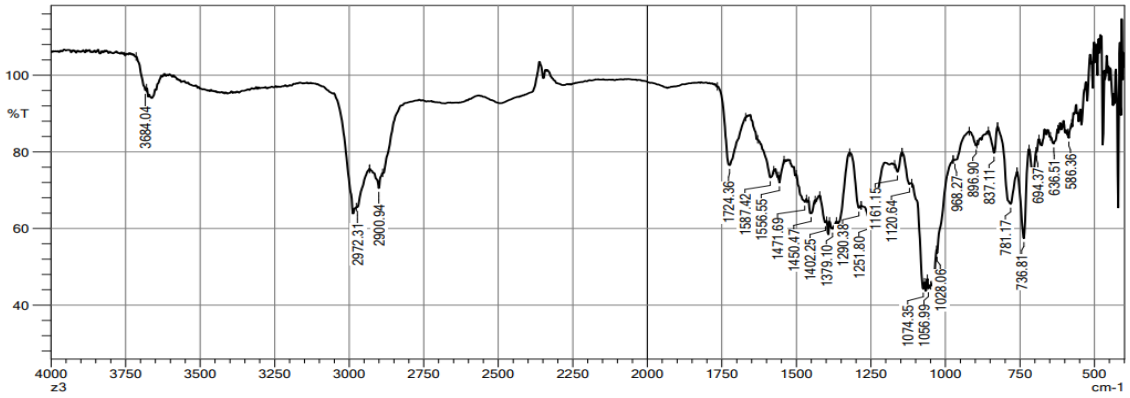


Figure (6): FT-IR spectra of (Z3).

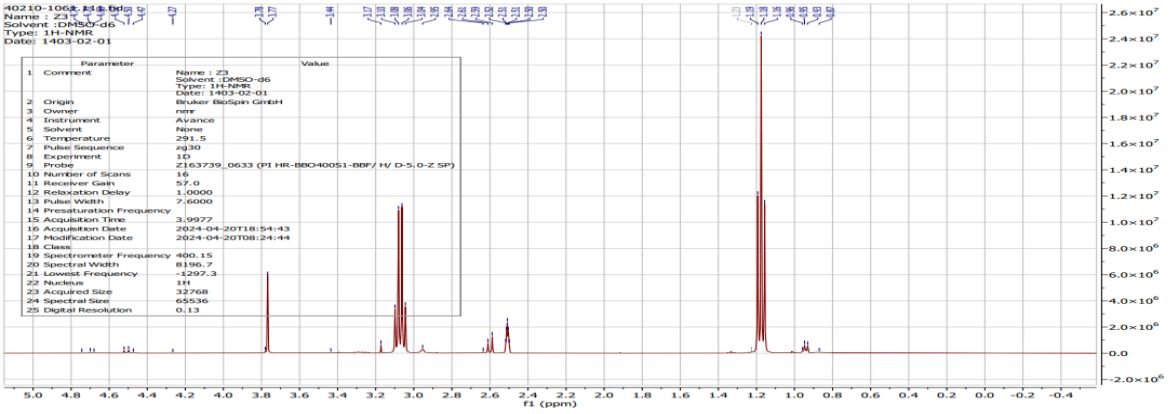


Figure (7): ^{1}HNMR spectrum of (Z3)

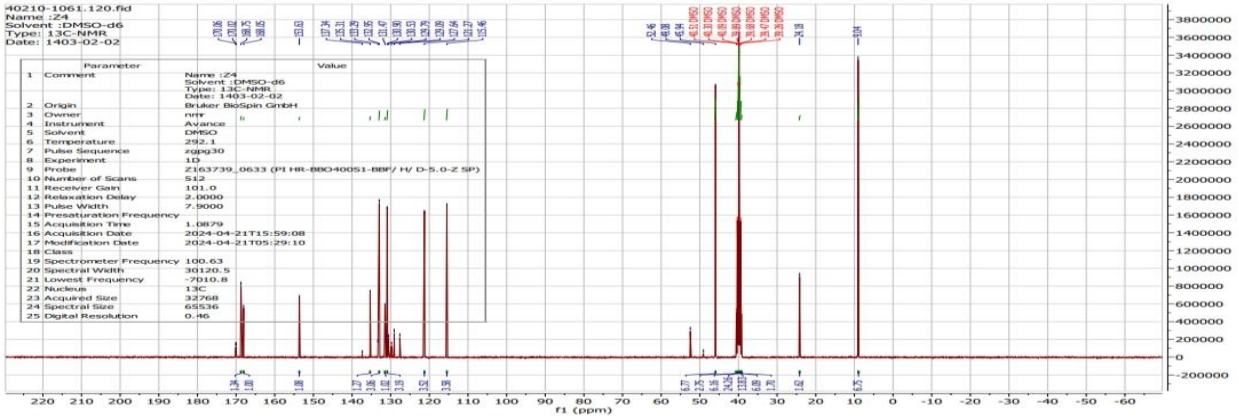


Figure (8): ^{13}C-NMR spectrum of (Z3)

3.3 Molecular docking study

3.3.1 Molecular docking of drug (Z3):

Table (5) shows the binding energy of the drug (Z3) with the amino acids present in the protein, as well as the minimum and maximum limits (RMSD). **Figure (9)** shows the association of the drug (Z3) with the amino acids contained in the protein of the lung cancer line. These acids include: the amino acid arginine (Arg), which has the serial number (C:196), as well as the amino acid threonine (Thr), which has two serial numbers. (B:170) (C:140), glutamic acid (Glu), which has the serial number (C:199), asparagine (Asn), which has the serial number (C:235), and the amino acid valine (Val), which has the serial number The sequence (B:172) is linked to the drug by a dark green hydrogen bond. . Likewise, a bond appeared in orange (Bi-anion) for glutamic acid (Glu), which has the serial number (B:171), and a bond appeared in purple (Bi-Skma) for the amino acid valine (Val), which has the serial number (B:172). As for the amino acids that appeared with a light pink bond called the (bay-alkyl) bond, they belong to both the amino acid lysine (Lys), which has the serial number (C: 139), and the amino acid alanine (Ala), which has the serial number (C). :138), as for the two amino acids, as well as glycine acid (Gly, which has the serial number (C: 199), and glutamic acid (Glu, which has the serial number (B: 171),) are linked by a carbon-hydrogen bond (C – H), which is very light green in color. As for the remainder of The amino acids that appear in light green when molecularly bound are bound to the protein with a van der Waals force.

(Thr – Ser – Leu – Phe – Met – Asp – His – Arg)

Table (5) displays the drug's binding energy (Z3)

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
1tup_Fragment_uff_E=895.47	-7.5	0	0.0	0.0
1tup_Fragment_uff_E=895.47	-7.2	1	14.165	19.041
1tup_Fragment_uff_E=895.47	-7.1	2	1.767	6.963
1tup_Fragment_uff_E=895.47	-7.0	3	1.873	8.765
1tup_Fragment_uff_E=895.47	-7.0	4	1.989	6.859
1tup_Fragment_uff_E=895.47	-7.0	5	14.252	19.14
1tup_Fragment_uff_E=895.47	-6.9	6	13.92	18.634
1tup_Fragment_uff_E=895.47	-6.8	7	1.667	7.338
1tup_Fragment_uff_E=895.47	-6.8	8	14.6	18.668

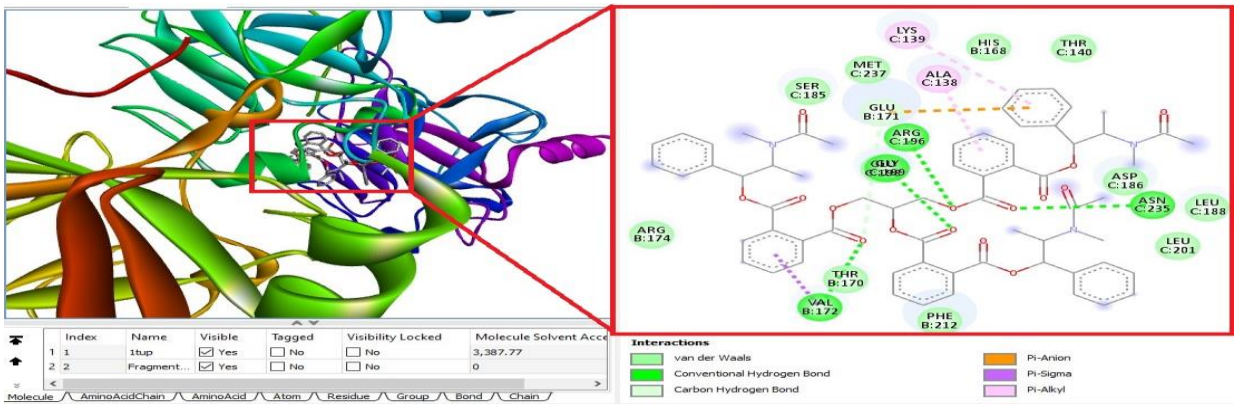


Figure (9) Shows the binding of the drug (Z3) to amino acids.

Anti-Cancer Measurements

In Figure 10 to 12, the effectiveness of the biological material, the effectiveness rate of the drug (N-acetylpseudoephedrine) loaded on the polymer, and the extent of its effect on inhibiting the activity of lung cancer cells are shown.

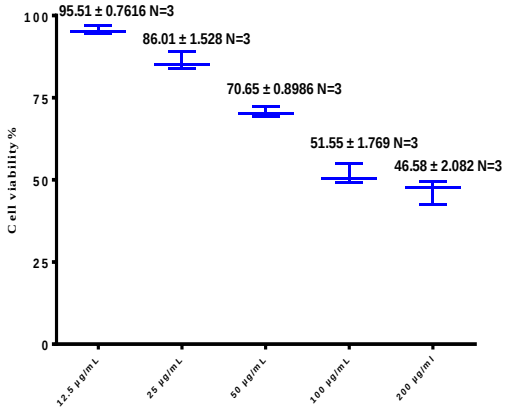


Figure 10: Cytotoxicity of z3 in A549 cells. IC50=30.258 µg/ml

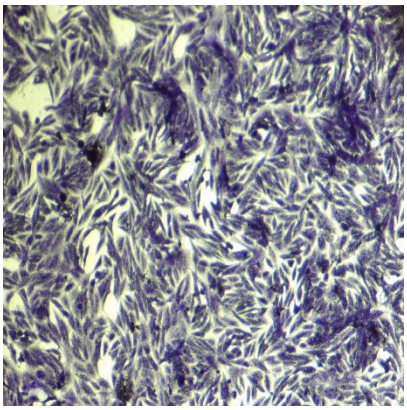


Figure 11: Control Un-treated A549 cells

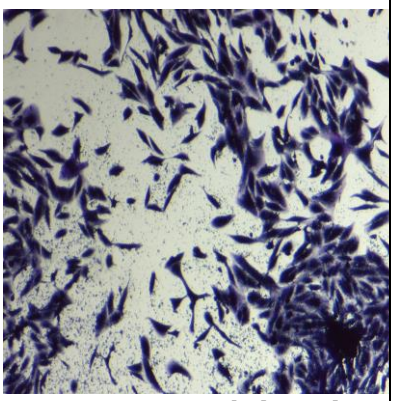


Figure 12: Morphological changes in A549 cells after treated with z3

conclusion:

The unique properties of drug-bound polymer nanocomposites make them useful in various biomedical applications. Regarding the effect of nano co-polymer linked to the drug (N-acetyl pseudoephedrine) on lung cancer cells. The loaded drug (N-acetylpseudoephedrine) was characterized on polymer nanoparticles using FT-IR, 13C-NMR, 1H-NMR AFM, and TEM. The effect of the prepared drug on lung cancer cells was measured. It was compared to chemotherapy used to treat cancer. These new medical nanocomposites have achieved impressive results in treating lung cancer cells.

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