Article

Treatment of Liver Cancer by Novel Nano Graft Co-Polymer-Amoxicillin Composite

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Abstract:

Nano Graft Co-polymer-Drug composite pharmaceutical were created by two-step reaction process; step one: 1.0 mole of glycerol and 3.0 moles of phthalic anhydride were reacted to create synthesis graft nano co-polymer, which was then characterized using FT-IR, 1H-NMR, AFM, and XRD techniques. Other step: To create a unique nano graft composite drug, this graft nano co-polymer was combined with distinct of medication, Amoxicillin. The resulting drug was then characterized using FT-IR, ^{1}HNMR, and ^{13}C-NMR spectroscopies. Using the software (PyRx and Biovia), the study of molecular docking was conducted to determine the binding position of the ligand of a protein with amino acids. The study examined the effects of the above-prepared composite drug on the HEp-2 liver cancer cell line. The results indicated that compound was highly effective in preventing the spread of hepatic cancer cells, as evidenced by the significant decrease in the percentage of these cells. Additionally, the cytotoxicity of a novel nano graft composite drug with amoxicillin hep-2 cells (IC50=36.932 µg/ml)

Keywords: Nano Graft Co-polymer, Liver Cancer, Amoxicillin, Cytotoxicity, Molecular Docking, Hep-2 Cells line

1. Introduction

A lot of interest has been shown in the functional polymers utilized in medical applications, which include dental, medical device, tissue engineering, artificial organs, and more [1, 2]. Because of this, the study of polymer identification that is employed as a therapeutic agent and the pharmacological characteristics of these

polymers can be helpful in serving as transporters of macromolecules like proteins or small molecules [3, 4]. Additionally beneficial and acceptable are synthetic polymers containing biological components [5]. By delivering medications to the site of action during the course of treatment, drug delivery systems help increase the safety and therapeutic efficacy of pharmaceuticals. Drug delivery systems may lessen dosage quantity and frequency, as well as side effects and biological inactivation [6, 7]. Pro-drugs made of biodegradable and non-biodegradable polymers are mostly used in injectable and implantable systems [8, 9]. One ideal carrier system for intravascular systems is one that circulates blood, is compatible with blood, does not deteriorate in vivo before or after drug release, and avoids excretion in the kidneys and the target area [10, 11]. Bioactive polymers basically provide a lot of advantages due to their high molecular weight and compatibility. To that end, polymers appear to offer a number of benefits as therapeutic agents compared to low molecular weight molecules [3]. Other benefits include reduced toxicity, greater specificity of action, and higher activity as a result of their interactions [12, 13].

Structural heterogeneity and the problem of molecular weight polydispersity are some characteristics that hinder the process's development [14, 15]. Low molecular weight medications have not been able to establish therapeutic profiles, while higher molecular weight polymers and their potentially limited pharmacological properties can be used to create and develop therapeutic agents for disease circumstances [16].

2. Methodology

* All the used chemicals were from analytical grade.

2.1. Synthesis of a Novel Nano Graft Co- polymer (S1)

3.0 moles of phthalic anhydride (27.5 g) and DMSO (7.5 mL) were combined in a 125 mL beaker. The mixture was then heated to 120°C, while being continuously swirled using a magnetic stirrer. A colorless liquid was formed as a result. To the mixture, glycerol (1.0 mol, 5.75 g) was added. The reaction flask is then filled with batches of about 12 mL of xylene to eliminate the water that is left over. The heater turns off after fifteen minutes. A solution is obtained, which is precipitated using cold deionized water 3.0°C. After filtering and rinsing with additional deionized water, the precipitate is left to dry at room temperature. The precipitate is then broken down to create the nano co-polymer.

2.2. Synthesis of Acid Chloride for a Novel Nano Graft Co-polymer (S2) [17]

After mixing (0.6 g) of S1 with (4-5) drops of thionyl chloride ($SOCl_2$) and (6.0 mL) of DCM in a 25 mL beaker with a magnetic stirrer, the mixture was permit to settle for 30 min at room temp before being kept at 65 degrees celsius for two hours. The nano graft co-polymer-acid chloride (S2) is prepared by drying out the solution for two hours and raising the temperature to 85 ℃.

2.3. Synthesis of a Novel Nano Graft Co-polymer- Amoxicillin Composites [18]

a mixture of Amoxicillin (1.5 g, 0.004 mmol) and (500 µL) of Et_3N was added to the previously prepared pharmaceutical ampoule, which contains 0.6 g of the compound (S2) and leaving it to heating at 85 °C, and then add 8 mL of DCM and cool using ice while stirring. The contents of the ampoule are poured into a 50 mL beaker filled with distilled water ice grits and stirred with a glass stirrer until the ice melts and the sediment is filtered out. Ethylamine is then left stirring in ice for an hour at room temperature for three hours.

2.4. Molecular Docking

Prior research indicates that the proteins listed in the [Protein Data Bank (PDB)] were chosen, and that of protein was discovered (1TUP) in the cancer cell. The PyRx program was used to attach the medication to the protein and displays the strength of the bond between the two. The program (BIOVIA) identified the binding locations, which clarifies how the medication attaches to the crucial amino acids that make up of protein chain. Along with providing two-dimensional images, it also describes the strength and length of the bindings that the medication has[19].

2.5. Biological activity

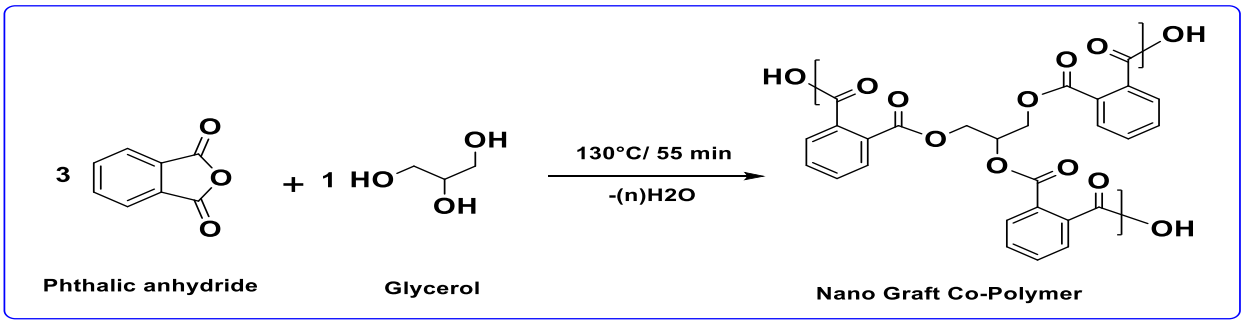
The HEp-2 cells viability MTT assay was carried out using 96-well plates to assess the impact of the produced copolymer drug graft complexes and the cytotoxic effect (Subjects x) on metastasis (liver cancer). The tests were conducted in accordance with the methodology described in the references [10, 20].

3. Result and Discussions

3.1. Synthesis of a Novel Nano Graft Co-Polymer (S1)

Condensation polymerization was used to create the nanoparticle-grafted copolymer (S1). Polymerization occurs 55 minutes after a reaction between 3.0 moles of phthalic anhydride and (1.0 mol) of glycerol at 130°C. Equation (1) describes the polymerization of the process and the subsequent release of water as a byproduct. FT-IR, ^{1}H-NMR, AFM, and XRD were then used to character and

describe this nano graft co-polymer.



Equation 1. Nano graft co-polymer (S1) reaction

Figure (1) displays the substance (S1) FT-IR spectra. It exhibits multiple absorption bands, including a broad band at (3075 cm^{-1}) that corresponds to the (OH) group of the alcohol and the hydrogen bond, and a band at (3005 cm^{-1}) that results from the vibration of the aromatic bond (C-H). In the FT-IR spectrum, the ester bond (C=O) is represented by an absorption band at (1669 cm$^{-1),}$ whereas the band at (1069 cm^{-1}) is connected to (C-O). ester. Two substitutions of the aromatic ring at (734 and 897 cm^{-1}) led to the development of two bands. The abnormal proton in the singlet signal for the carboxylic acid group at 13.04 ppm is explained by the ^{1}H-NMR spectra displayed in **Figure (2)**. A multiple of methyl protons at 4.15 ppm, the absence of an aliphatic alcohol signal, signals for four methylene protons in the co-polymer structure at 4.26–4.28 ppm, and a multiple in the region 7.55–7.68 ppm attributed to phthalic anhydride are indicators that a nano graft co-polymer is forming.

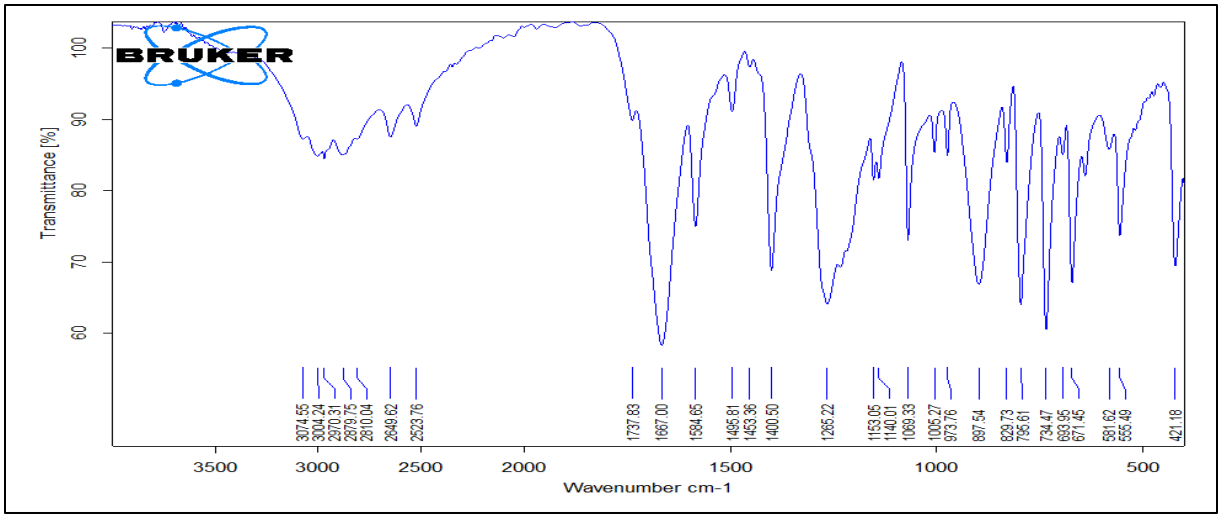


Figure 1. FT-IR spectra of the Nano graft Co-polymer (S1).

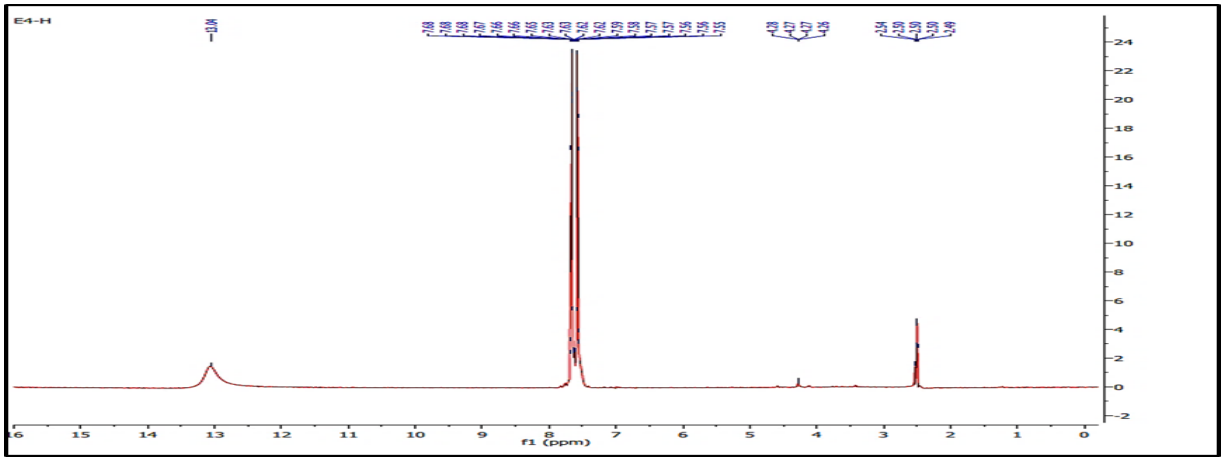


Figure 2. ^{1}H-NMR spectra of the Nano graft Co-polymer (S1)

The exterior of the co-polymer nanoparticles is seen in Figure 3(a, b) of the AFM. The Co-polymer surface's square root square was 5.94 nm, and its roughness coefficient was 5.08 nm. This suggests a significant relationship between the large size of the nanoparticles and the homogeneous crystalline structure, surface roughness, and homogeneity. Furthermore, as Figure 3a shows, the average particle height was equal to 22.04 nm. The findings indicate that the co-polymer (S1) nanoparticle's molecular size was 68.62 nm. Table (1) displays the ratios of the different volumes as well as the overall rate of the most common nanoparticle particle sizes.

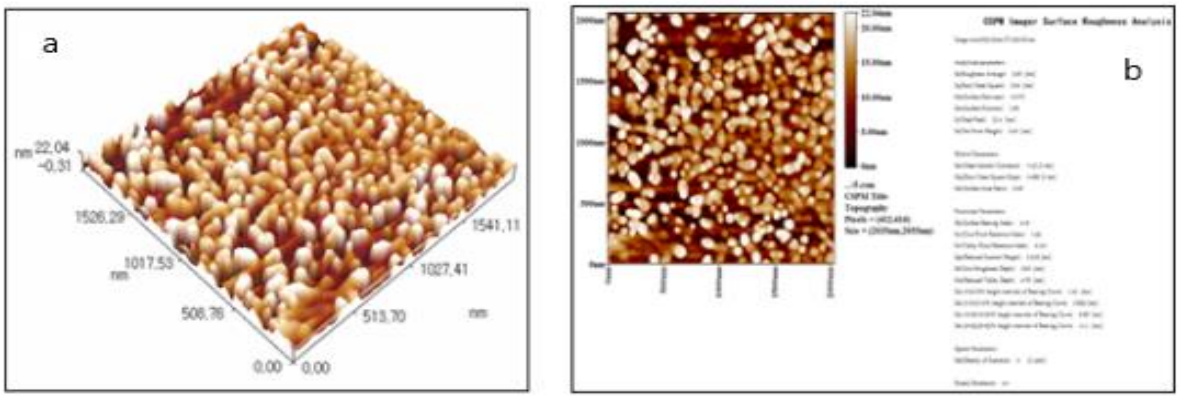


Figure 3. a: 3D image is displayed by the Atomic Force Microscope for nano co-polymers.

b: Two-dimensional image is displayed by the AFM image for nano co-polymer.

Table 1. Displays the overall rate of the nano co-polymer nanoparticle particle sizes together with the relative proportions of the various volumes.

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			

As seen in Figure (4), the nano polymer's x-ray diffraction (XRD) patterns revealed a diffuse halo at 2q = 20, which is connected to the intra-chain segment distance. Every polymer sample exhibits more unique peaks in its diffractogram. In contrast to polymers that are only aliphatic, the XRD pattern shows that stiff aromatic rings formed from phthalic acid lead to more rigid structures, which should result in a higher potential for crystallization. The polymer synthesis was carried out with a phthalic acid to glycerol molar ratio that produced a higher concentration of carbonyl groups. Increased phthalic acid concentrations also implied that molecular motions brought on

by the stiffness of aromatic rings might aid in the ordering of polymer chains in crystalline lattices. Figure (4) illustrates how to use Origin software to obtain x-ray diffraction (XRD) for the co-polymer of nanoparticles. The aforementioned AFM measurements are consistent with the average inters planer distance (d_{hkl}) of 0.416 nm between atoms, as determined by Bragg's Law.

$$n\lambda = 2d\sin\theta \ldots\ldots\ldots \text{ Bragg's Law}$$

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

$$D = \frac{k\lambda}{\beta\cos\theta} q \quad \ldots\ldots\ldots \text{ Scherrer's Equation}$$

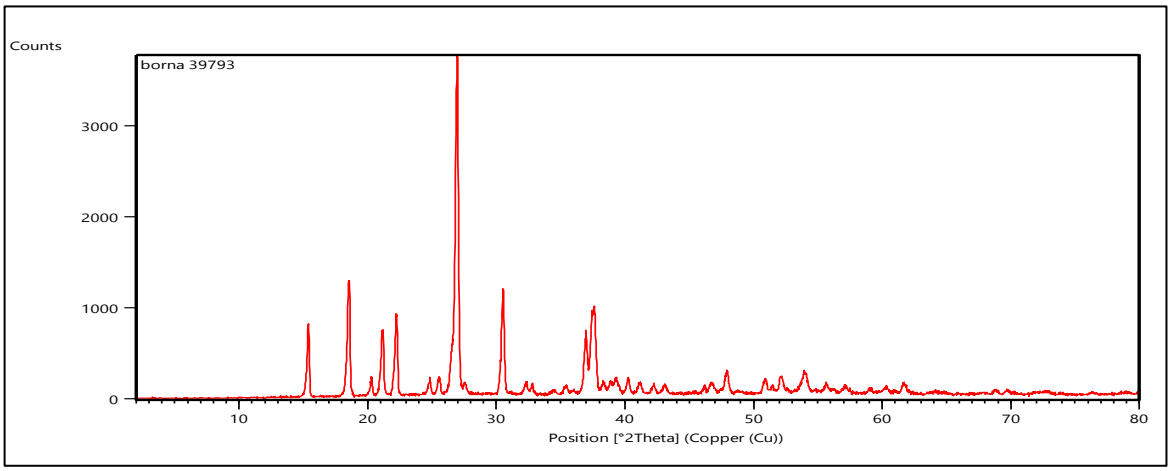


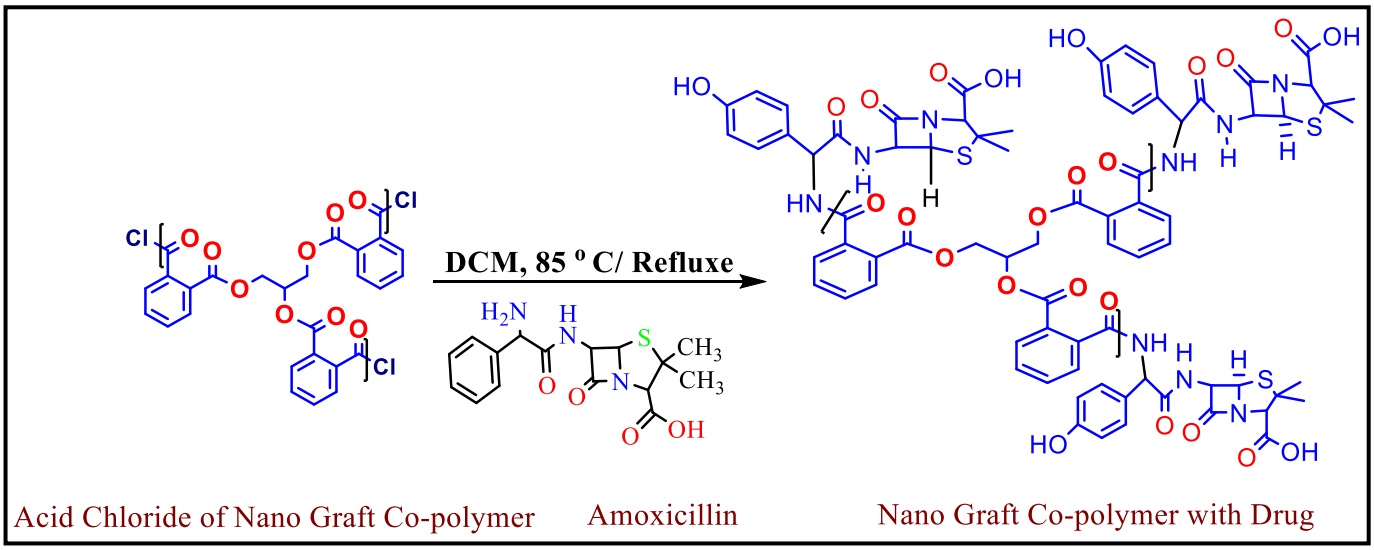
Figure 4. X-ray diffraction of Nano graft Co-polymer

Table 2. Lists the proportions between the crystallite diameters and the nano co-polymer's "d-spacing or atom-to-atom 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 a Novel Nano Graft Co-Polymer-Drug Composite (S3)

About (0.6 g) of compound (S2), that reacted with (3.5 g, 0.009 mol) Amoxicillin drug and was combined to collect was added to a mixture of half mL trihydrate and 8 ml DCM at 85°C. Equation (2), represents the reaction.



Equation 2. Reaction of the Composite (S3)

Compound (S3) FT-IR spectrum is displayed in Figure (5), where an absorption band appears at (3404.36 cm^{-1}) and is attributed to the bond (O-H) carboxyl group, (-C=O-OH) carboxyl, and absorption bands of C-Haliph at (2985.81 cm^{-1}), C=O β-lactam at (1784.15 cm^{-1}), C=O-N amide at (1666.5 cm^{-1}), and absorption bands of C=C at (1565.55 cm^{-1}), C-O at (1269.16) cm^{-1}, and C-N at (1215.72 cm^{-1}), respectively.

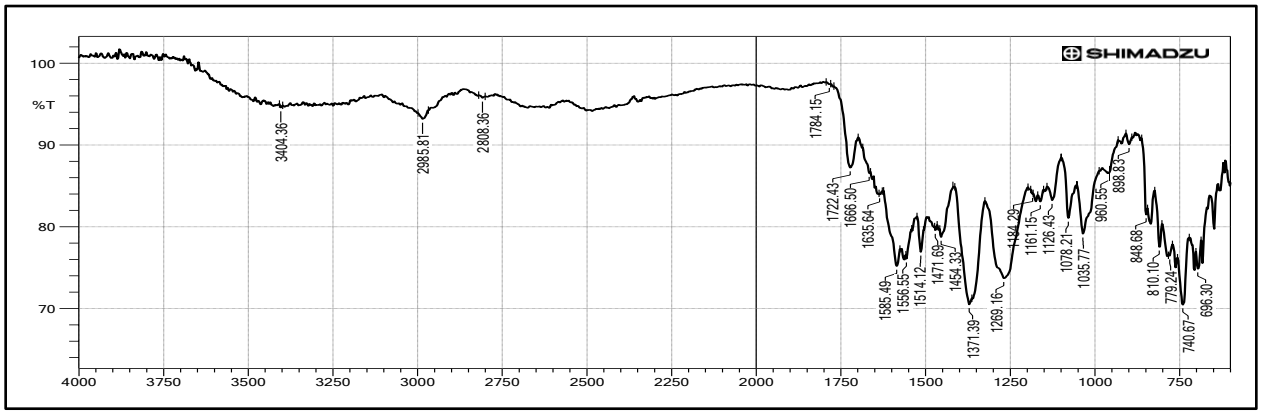


Figure 5. Compound (S3) FT-IR spectra

Figure (6) shows the ^{1}H-NMR spectrum of the compound (S3), where appearance signal of the hydroxyl that attached to the benzene ring of drug at 7.35 ppm and appearance and appear signal of the H of drug secondary amine at 7.25 ppm and appearance signal at 5.77 ppm for aromatic hydrogen, and appear signal for 1H for C-H at 3.72 ppm and appearance signal for Hydrogen of CH near S-bond at 2.98 ppm. ^{13}C-NMR spectrum of the compound (S3), **Figure (7)** shows the presence of a signal of carbonyl amide at 133.8 ppm and a signal of 133.18 ppm for carbonyl of secondary amide, and a signal appeared at 129.11-127.29 ppm for carbons of drug aromatic ring and a signal appeared at 40.31 ppm for carbon near carbonyl amide, a signal appeared at 40.10 ppm for carbon near S-bond.

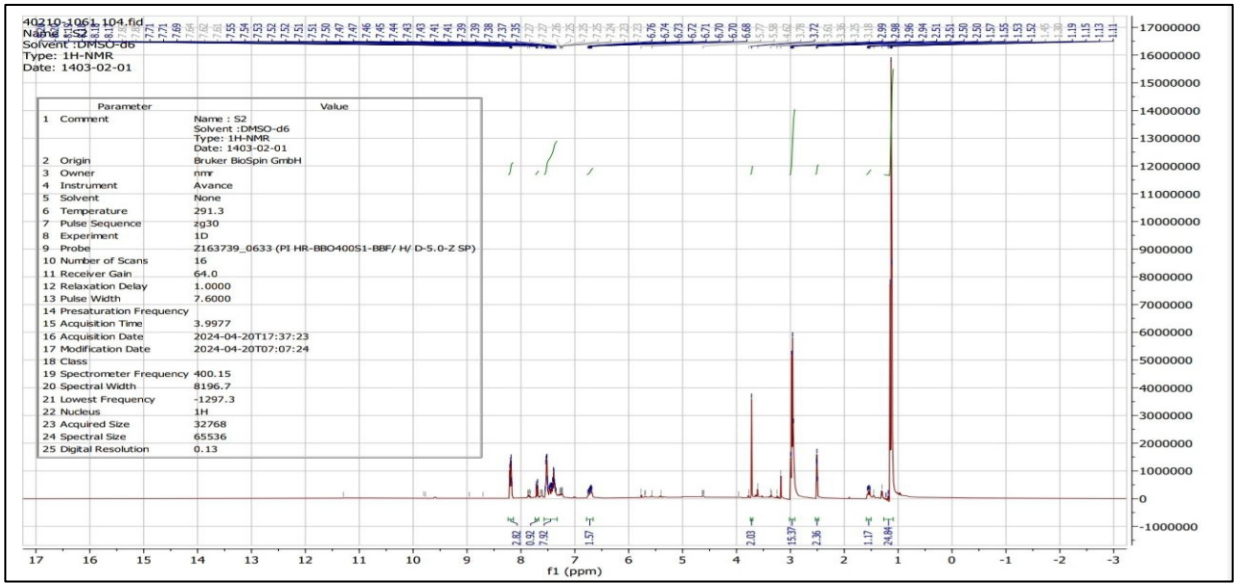


Figure 6. Compound (S3) ^{1}H-NMR spectra

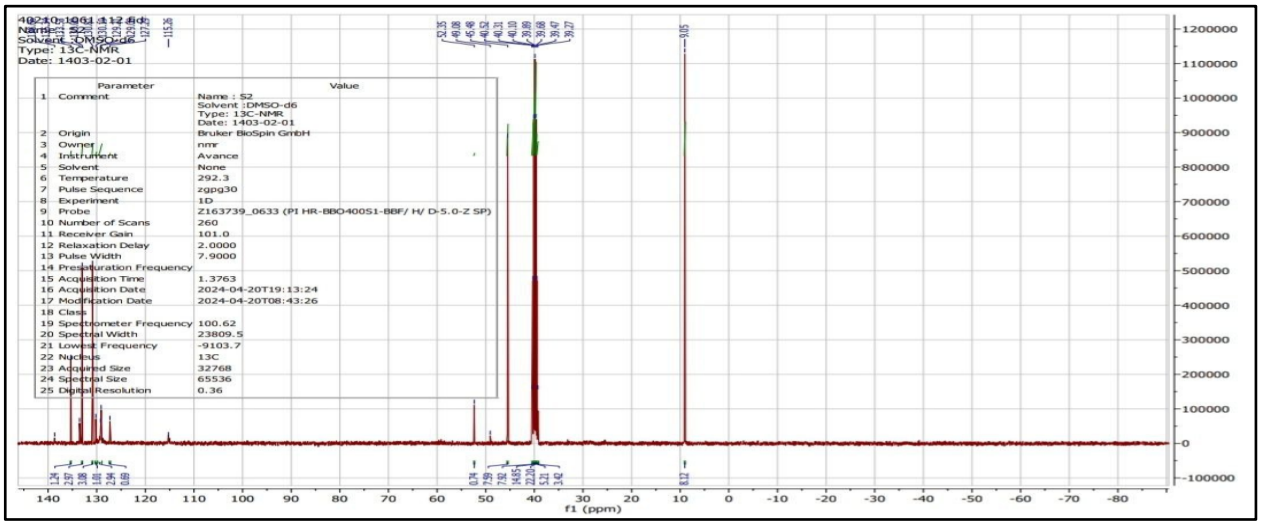


Figure 7. Compound (S3) ^{13}C-NMR spectra

3.4. Molecular Docking Study

Binding energy of the drug (S3) with the amino acids present in the protein (**Table 3**), as well as the minimum and maximum limits (RMSD). Figure (2) shows the binding of the drug (S3) with the amino acids contained in the protein of the liver cancer line, and these acids are: the amino acids and cysteine (Cys) which carries the serial numbers (I:58, I:12), and glycine (Gly) which carries the serial number (A:219), and aspartic (Asp) which carries the serial number (I:10) which are linked to the drug by a hydrogen bond in dark green. Also, a yellow (pi-sulfur) bond appeared for cysteine (Cys) which carries the serial numbers (I:22, I:14, A:191, A:220). As for the amino acids that appeared with a light pink bond called a (pi-alkyl) bond, they belong to the amino acid lysine (Lys) which carries the serial number (A:224) and the amino acid proline (Pro) which carries the serial numbers (I:61, I:20). As for the amino acid aspartic (Asp) which carries the serial number (I:10), it is linked to a carbon-hydrogen bond (C-H) which is very light green. As for the remaining amino acids that appeared in light green when they are molecularly bound, they are linked to the protein by van der Waals force.

(Tyr – Ser – Pro – Asn – Cys – Gln – Phe – Glu – Gly)

Table 3. Shows the binding energy of drug (S3)

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
1d6r_Fragment_uff_E=1978.53	-7.2	0	0.0	0.0
1d6r_Fragment_uff_E=1978.53	-7.1	1	3.778	10.469
1d6r_Fragment_uff_E=1978.53	-6.9	2	4.305	7.329
1d6r_Fragment_uff_E=1978.53	-6.8	3	3.357	9.103
1d6r_Fragment_uff_E=1978.53	-6.8	4	3.615	6.861
1d6r_Fragment_uff_E=1978.53	-6.8	5	2.895	7.394
1d6r_Fragment_uff_E=1978.53	-6.8	6	3.415	9.589
1d6r_Fragment_uff_E=1978.53	-6.7	7	3.631	10.547
1d6r_Fragment_uff_E=1978.53	-6.7	8	15.296	20.652

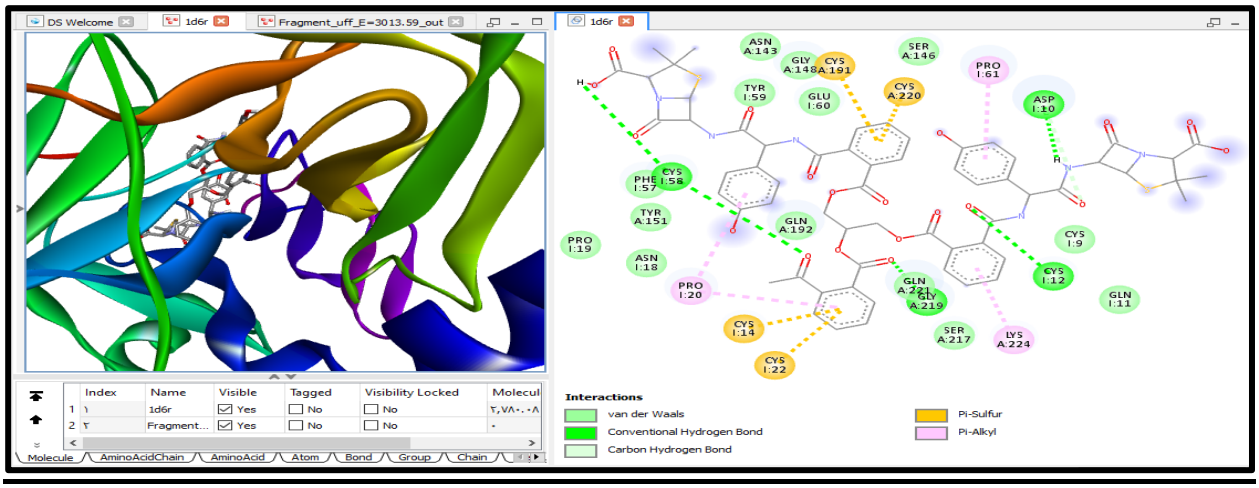


Figure 8. Shows the association of drug (S3) with amino acids

3.5. *Biological Activity*

HEp-2 cells were used as the test subject for the cytotoxicity of nano graft co-polymer-Amoxicillin (S3) at various concentrations (12.5, 25, 25, 50, 100, and 200). After incubation for 72 hours, the impact of (S3) on the viability of HEp-2 cells was assessed. In contrast, cell viability was considerably reduced in a dose-dependent manner by the other concentrations. 36.932 µg/ml is the IC_{50} value of the Nano Graft Co-polymer Amoxicillin in HEp-2. Using 200 µg/ml (S3) NPs, the majority of cells were eliminated. **Figure (9)**

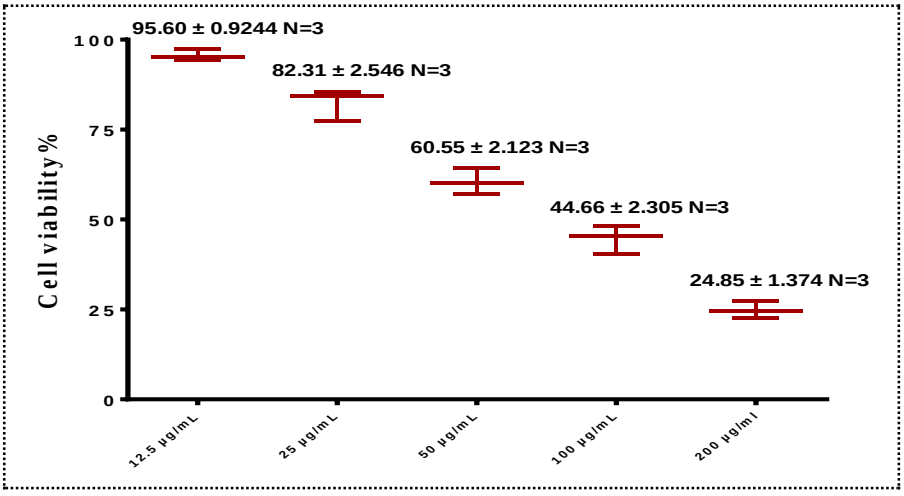


Figure 9. Cytotoxicity of S3 in HEp-2 cells, IC50=36.932 µg/mL

Conclusion

FT-IR, ^{1}HNMR, AFM, and TEM were the techniques used to synthesis and characterize the grafted Nano Co-polymer. Novel drug nano composites were generated by chemically bonding drugs that are known to be employed as therapies for other diseases to the prepared graft Nano Co-polymer. These novel drug nano composites were utilized to stop the fast spread of liver cancer and were compared to chemotherapy, which is known to be a weapon against cancer cells. The new nano composites were characterized using techniques such as FT-IR, ^{1}HNMR, and ^{13}CNMR. When used to treat liver cancer cells, these novel nano-drug combinations have shown remarkable outcomes.

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