Sumer Journal for Pure Science (ISSN: 2790-7031)
2nd International Scientific Conference on Pure and Medical Sciences/University of Sumer 2024

Chemical Loading of Doxorubicin onto Onion DNA and its Medical use

Firyal Mohammed Ali[1] and Raisan Kadhim Taresh[2]

[1]*Al-Mustansiriyah University; Collage of Science; Department of Chemistry.Baghdad. Iraq*
[2]*Department of Chemistry, College of of Education , University of sumer, Al- Refaee , Thi –Qar :64005.Iraq*
Corresponding author: Drfiryal55@Gmail.com

Abstract

A new natural biopolymer was prepared by modifying the structure of the drug Doxorubicin by loading it onto DNA extracted from onions and dotted with Maleic anhydride monomer The goal of this design is to increase effectiveness of the anti-cancer drug known to be used medically, and to reduce the harms and side effects, the use of Doxorubicin may lead to the occurrence of many side effects, such as: cardio toxicity, bone marrow inhibition, and liver damage. It is also characterized by its anti-cancer activity and its status as a natural polypeptide. Its main chain was copolymerized using anhydrous malic acid. Then replace it with the amino drug Doxorubicin. In order to upsurge the drugs life, and release the modified drug with expanded drug action by gradual release with its excellent performance. This new biological polymer was applied to mice, and it gave distinct results. The infected mice were treated and made a complete recovery without secondary damage and without leaving traces. It could be a better drug than Doxorubicin if it was alone, by filling it onto the DNA. In order to gain thermal stability during storage. Compound RA has high selectivity and can be adopted as a drug for cancerous tumors and the option of its use and development, as it gave double distinct results without any effects on living cells.

التحميل الكيميائي للدوكسوروبيسين على الحمض النووي للبصل واستخدامه الطبي

فريال محمد علي [1] و ريسان كاظم طارش[2]

[1] الجامعة المستنصرية ; كلية العلوم ; قسم الكيمياء
[2] جامعة سومر ; كلية التربية ; قسم الكيمياء

المستخلص

تم تحضير بوليمر حيوي طبيعي جديد DNA-g- malic doxorubicin عن طريق تعديل بنية دواء الدوكسوروبيسين doxorubicinبتحميله على DNA المستخلص من البصل و المطعم بمونومرات المالئيك اللامائي. ينطوي هدف هذا التصميم على زيادة فعالية الدواء doxorubicin المضاد للسرطان المعروف باستعماله طبيا, ولتقليل المضار والاعراض الجانبية فقد يؤدي استخدام دوكسوروبيسين إلى حدوث العديد من الآثار الجانبية، فقد تم اختيار ا(لدي ان اي)المستخلص من البصل الذي يمتاز بفعاليته المضادة للسرطان ايضا و اعتباره بولي بيتايد طبيعي فقد تم اجراء البلمرة المشتركة على السلسلة الرئيسية له باستخدام حامض المالئيك اللامائي. ثم تعويضه بالدواء الاميني . doxorubicin ولأجل زيادة عمر الدواء , و تحرير الدواء المعدل مع العمل الدوائي الموسع عن طريق الإطلاقات التدريجية بأدائه الممتاز. تم تطبيق هذه البوليمر البيولوجي الجديد على الفئران ، وأعطى نتائج متميزة وتم علاج الفئران المصابة وتماثلها للشفاء الكامل دون حدوث اضرار ثانوية و دون ترك اثار و يمكن ان يكون دواء افضل من دواء الدوكسوروبيسين لو كان لوحده, وذلك بتحميله على DNA المطعم . ولأجل اكتساب ثباتيه حرارية اثناء الخزن و يمتلك انتقائية عالية و يمكن اعتماده كدواء للأورام السرطانية وامكانية استخدامه وتطويره حيث اعطى نتائج متميزة مضاعفة نسبة الى الدوكسوروبيسين دون حصول اثار جانبية على الخلايا الحية.

1.Introduction

The good improvements of some natural polymers to their derivatives have been applied [1], as widely used in pharmaceutical and biomedical preparations, and many polypeptides have been widely used to regulate drug release and many delivery systems as they have many compensations, compared to their synthetic polymers, because they are less

luxurious [2] biodegradable, Non-toxic,and readily available. Drug-bound polymers typically display long half-lives, improve bioavailability, of drugs by plummeting their hydrolysis rate and increase the solubility, higher stability, lower immunogenicity, specific targeting to cells or tissues, improved cellular uptake, and permitting targeting and control of drug release. and lessen side effects [3].

In order to improve drug efficiency, many drug delivery systems have been designed to stretch and release multiple drugs in a controlled manner, such as the method of grafting polymer on the side functional groups on catalytic side chains [4]; Thus, copolymerization is a commonly used method for modifying polymer structures. Using a free radical initiator [5]. The effect of the reaction variables and their ratios on the water absorption capacity was studied [6]. DNA was first remote in 1869 from white blood cells by Swiss physician Friedrich Miescher. [7].DNA chain is called a polynucleotide and it is made up of simpler units called nucleotides [8].There are also methods for extracting DNA from various sources. These include food samples, formalin, urine, and tissues. Isolation of pure, intact, and high-quality DNA is critical for any molecular studies, by excessive contamination by secondary metabolites. DNA separation methods must be modified for both plant species and even due to the presence of these metabolites [9].

The search for a more effective method for extracting quality, yield-oriented DNA has led to the development of several protocols for the isolation of DNA from plants containing high levels of secondary metabolites [10]. And extra secondary metabolites such as flavonoids and alkaloids that hinder DNA extraction [11, 12]. Several factors can cause DNA to be cut during extraction. It straight or circuitously interferes with enzymatic reactions [13]. Polysaccharides may be particularly problematic when present in DNA samples, as their presence may also constrain enzymatic activity. It has been shown that the presence of polysaccharides inhibits the activity of the restriction enzyme [14] and the DNA sample with the formation of a very viscous solution [15]. “nuclein” was also used in addition to the general methods practical to extract DNA from many different tissues or cell types, there are also methods for extracting DNA from different sources [16]. Plasmid-improved green fluorescent protein transfer [17, 18]. Photochemical evaluation of poly(D, lactide-co-glycolide) [19]. Enhanced properties of dissociated lung-loaded PLGA nanoparticles during encapsulation and activity will power [20]. Optimization of the formulation of loaded PLGA nanoparticles by dual factor design and evaluation [21]. A nano medical system for DNA drugs shaped using a biodegradable nanoparticle stage [22].

2. Experimental and Materials

The DNA was extracted from onions, Doxorubicin was bought from the pharmacy, Ceric Ammonium Nitrate (CAN), anhydrous Maleic acid, Ether and Dioxane were acquired from Sigma company, all used chemical. Reagents were used without purification.

2.1. Instrumentation

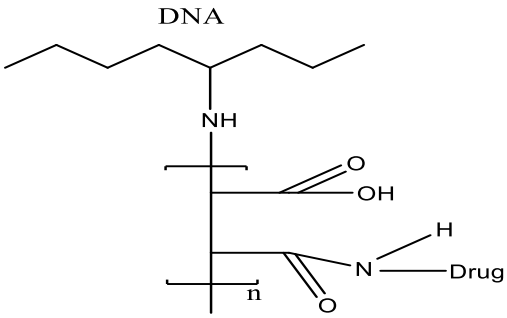
Melting point was measured by using a thermal microscope (Kofler method), Stuart SMP 30. Infrared spectrophotometer measurements were performed with Shimadzu FT-IR 8400 series Fourier Transform. DSC thermal analysis was done by NET Z Using a VARIAN (UV-Vis)-100 Conc UV-Vis spectrophotometer at room temperature.

2.2. Preparation of DNA Grafted Maleic Anhydride (RA)

Preparation of doped onions DNA with Maleic anhydride (RAD) With a weight ratio of 1:1,The copolymerization of DNA was performed. With Maleic anhydride as follows (1 g) onions DNA is dissolved In (5 mL) Dioxin, (1 mL) Ceric Ammonium Nitrate (CAN), ((1 g) Maleic anhydride, the mixture was introduced into a polymerization bottle, the mixture was animated for about (30) minutes at (40°C) , solvent was evaporated under reduced pressure, The yellow product was obtained with conversion ratio (56.15%) dp (110-115)°C .

2.3 Substitution of (RA) with Amino Drug (Doxorubicin)

DNA-g- Malic Doxorubicing DNA-g-malic anhydride was additional in (4 mL) Dioxin, (0.025 g 0.0062 M) Doxorubicin dissolved in (4 mL) Dioxin, then the mixture was heated for about (1 hour) at 30°C, the stained solution was filtered, the filter was isolated and the obtained solvent was evaporated, the product (RAD) of DNA-g-Malic Doxorubicin, the product was also dried at room temperature and the conversion ratio was calculated (85%), melting point ranged (140-150°C).



Drug =

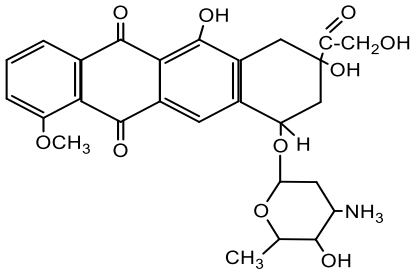
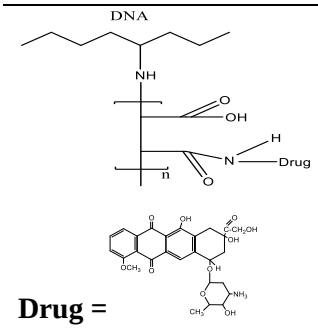


Table 1- Physical properties of prepared (RAD).

RAD	Color	Conversion ratio	Decompose point ºC	[η] dL/g
 **Drug =**	**Red**	**85%**	**140-150 ºC**	**0.012**

2.4 Determination of molecular weight of produced (RAD) using the Mark Hounk equation

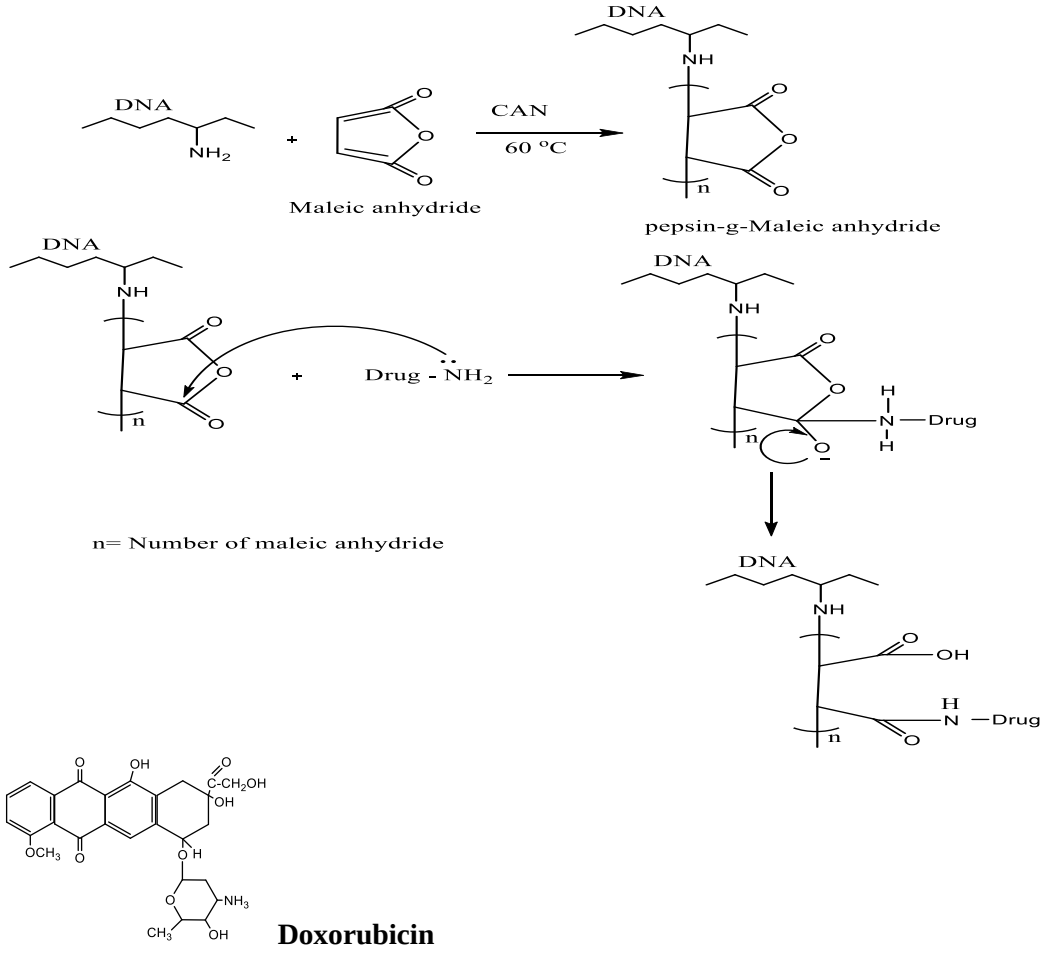
$(\eta) = K M^a$, where (η) is the intrinsic- viscosity which was determined using a capillary viscometer (KPG Cannon–Fenske type) , M is the average molecular- weight of the polymer, and K and a are constants that depend on the polymer,solvent system used. It measured 0.012 dL/g.

2.5 Controlled drug release

Release was studied by adding (RAD) 100 mg continuously in solution (100 ml).And with different pH values (1.1-7.4) at 37°C
The wavelength λ_{max} was measured at different intervals using an ultraviolet (spectrometer).
The sample was periodically analyzed by UV spectroscopy
UV rays were measured by the mole fraction of the drug sustained release over successive periods of (DNA) for onions. Which also has anti-cancer activity and the use of anhydride Maleic graft as an unsaturated monomer using the starter ceric ammonium nitrate, which is a linker between the drug and the DNA chain.

3. Results and Discussion

The goal of this design is to inoculate the modified drug Doxorubicin with extended pharmacological action by gradual release with its excellent performance. The application of this new biological polymer to the infected mice, and it gave distinct results. The infected mice were treated and cured at a higher rate compared to the original drug. And without secondary damage and without leaving side effects and increasing the drug life, and it can be a better drug than Doxorubicin if it was alone by loading it on DNA Doxorubicin B (DNA) by anhydrous Maleic linkage and using the grafted polymerase technique.
The natural biopolymer (DNA) and the inoculation of (DNA) extracted from onions, known for their high quality, were prepared by developing a new protocol by inoculating Doxorubicin by loading it on the DNA extract of onions. Which also has anti-cancer activity and the use of anhydrous Maleic vaccination as an unsaturated monomer using the starter Ceric Ammonium Nitrate, which is a linker between the drug and the DNA chain.



Mechanism -1 of ring opening reaction of DNA-g-Maleic drug by nucleophilic reaction

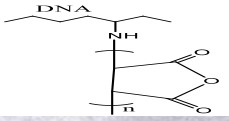
To acquire new properties of increasing solubility, decreasing crystallization and susceptibility to chemical changes due to the presence of important and basic active groups and will preserve the original physical and biochemical properties and will finally bring new or improved properties.

The products were characterized by infrared wavelength spectroscopy. Figure 1 and 2 show (in addition to the characteristic DNA peaks) in the grated DNA spectrum, there are some new absorption peaks. The peaks at 1740, 1537, 1450, 1392 cm-1 are the -CO group, -COO group, -CH group and -COO group, respectively..

Large absorption bands between 1200 and 1000 cm-1 were observed and credited to the free amine NH2). The peak at 1624 cm−1 can be based on the C-N vibrational stretching bound to the OH group by bonding and the adsorption band at 1377 cm−1 represents the C-O stretch of the initial alcohol group. The bands at 1100, 1060 and 1024 cm-1 showed a stretching of the C-O bond. The intensity in the region (3000–3400) cm-1 attributed to intermolecular and intermolecular H bonds indicates a decrease in these regions in agreement with the intensity observed in the region (1660-1600) cm-1, (1705 cm-1) due to (C = O) carboxylate, another band appears at (1602 cm−1), due to (NH) amine bending.

Figure (2) FTIR spectrum of DNA-g-Maleic Doxorubicin showed the absorption profile at (3371 cm−1), indicative of the presence of (OH, group), and (2852,2924 cm−1), due to

(aliphatic CH), (1705 cm−1) due to (C = O) carboxylate and (1633 cm−1), due to (C = O) amide, (1219 cm^{-1}) due to phosphine oxide.



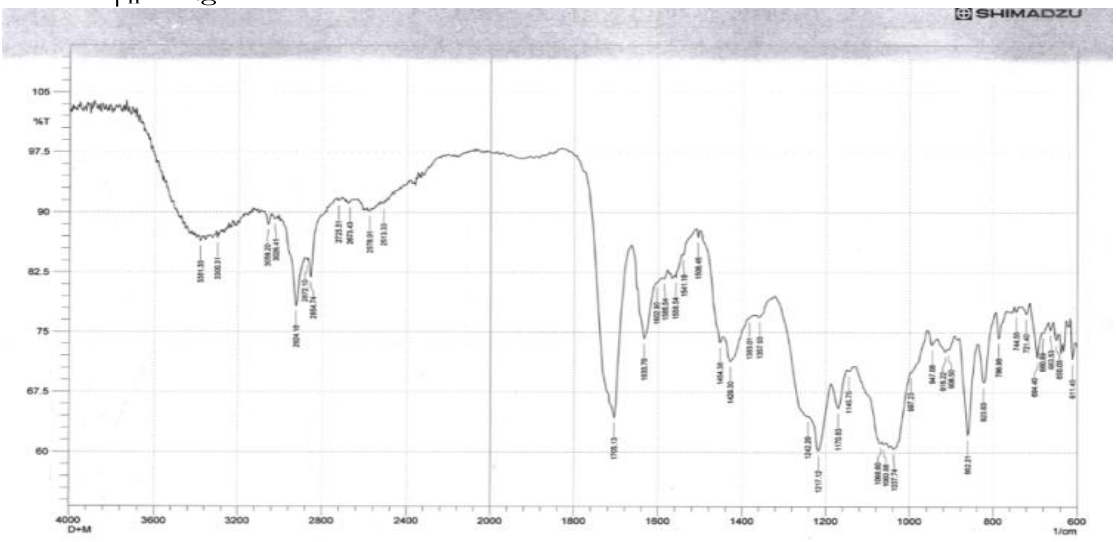


Figure -1 FTIR Spectrum of polymer DNA-g-Maleic anhydride

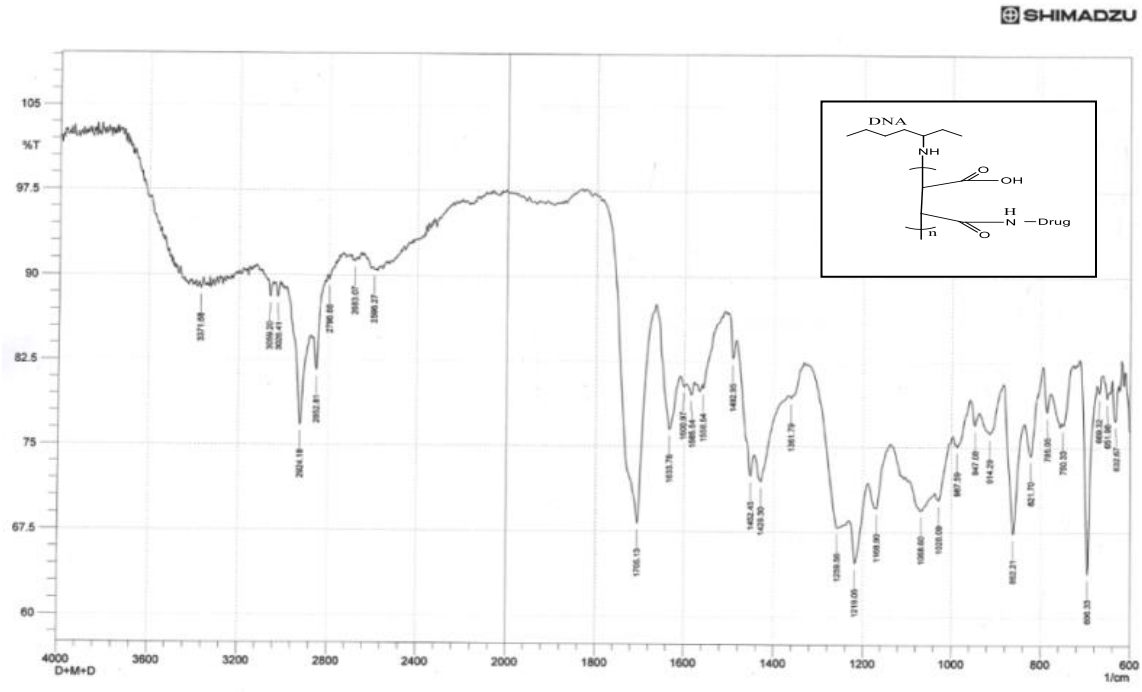


Figure-2 FTIR Spectrum of polymer DNA-g- Doxorubicin Maleic anhydride.

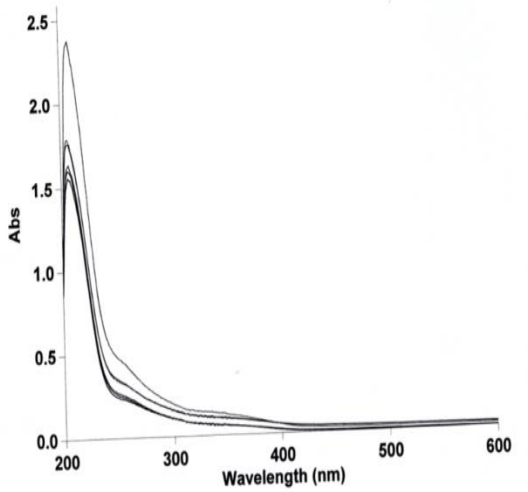


Figure -3a UV Spectrum of basic hydrolysis of RAD in pH7.4 Controlled drug release

The release of (RAD) was studied at different pH values (1.1-7.4) at 37°C, The λmax wavelength was measured at different intervals using an ultraviolet spectrophotometer. The drawn sample was occasionally analyzed by UV spectroscopy and quantified by the mole fraction of the drug released at successive time intervals. Figure (3) shows typical features of the hydrolysis, and the total release of the active. compound was reached to a greater degree in the basa medium.

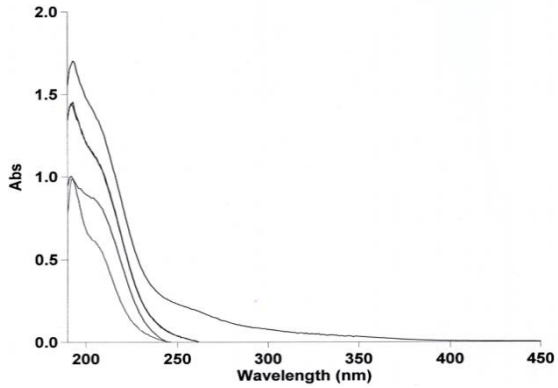


Figure -3b UV Spectrum of acdic hydrolysis of RAD in pH1.1.

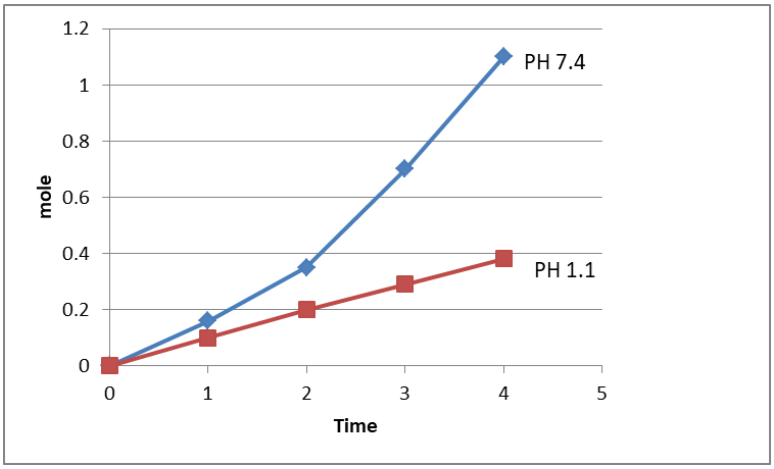


Figure (4) hydrolysis RAD in PH 7.4 and pH 1.1

Sumer Journal for Pure Science (ISSN: 2790-7031)
2nd International Scientific Conference on Pure and Medical Sciences/University of Sumer 2024

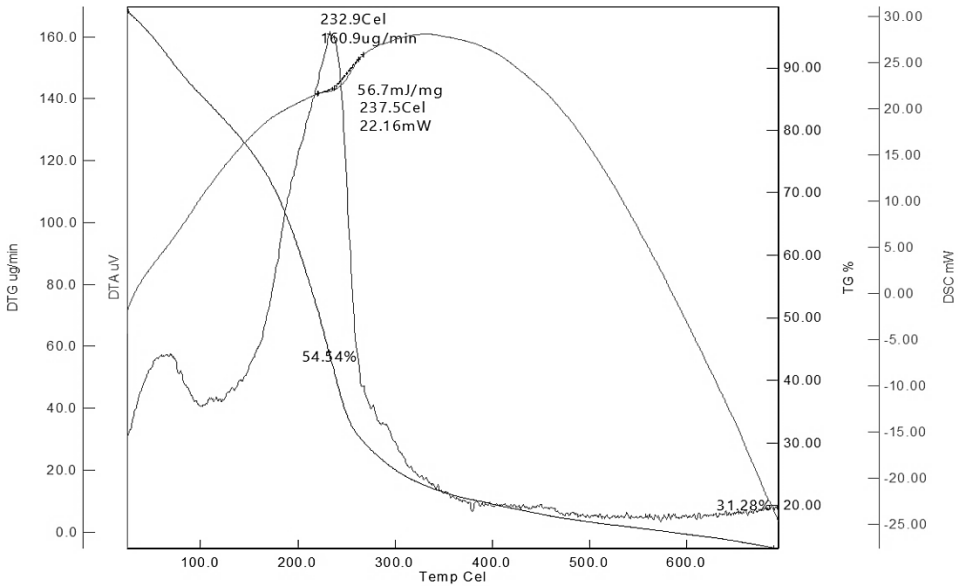
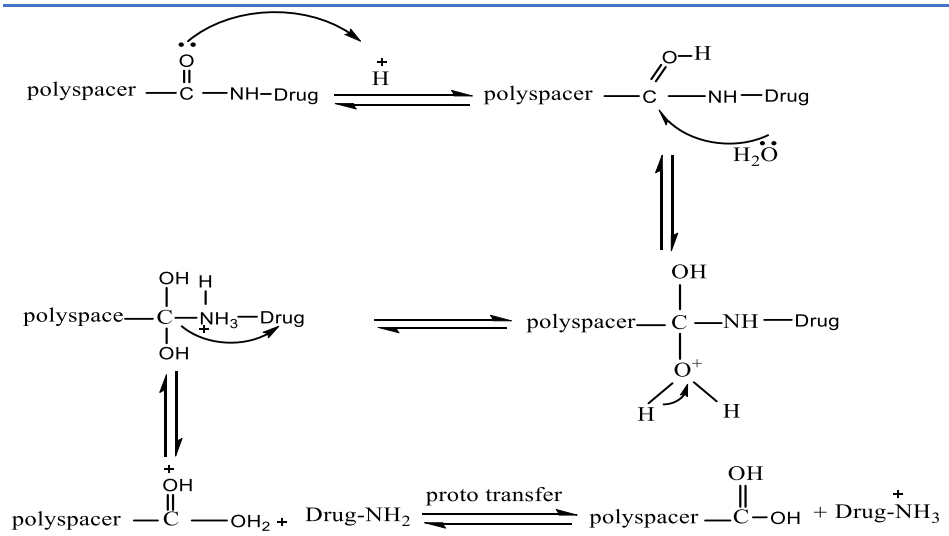


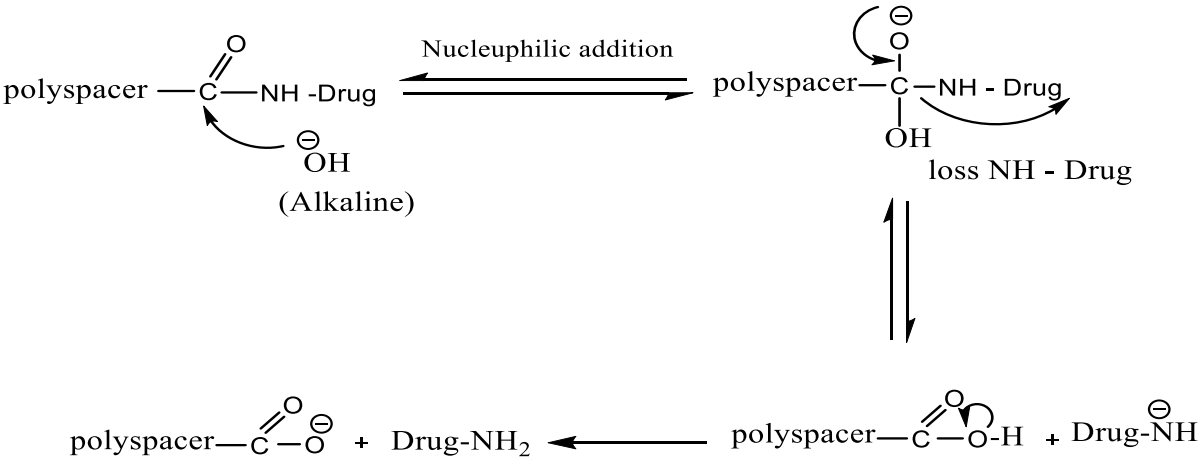
Figure -5 TGA and DSC of RAD

Thermal analysis showed Figure (5) for an endothermic peak appeared at 90°C. Two peaks were observed at 100°C and 110°C, which indicates a heat peak that may result from the decomposition of the amide bond. TGA in (two-stages weight loss. Amount of weight: 40 °C and 100 °C shows about 65% of the weight loss. This may correspond to a 10% loss of adsorbed water in weight.

The second phase of weight loss begins at 90°C and continues up to 110°C, during which there was a 55% weight loss due to glycolysis Less weight than copolymerDNA.



Scheme -2 Mechanism of hydrolysis Doxorubicin-DNA in acidic medium



Scheme -3 Mechanism of hydrolysis Doxorubicin-DNAin basic medium

This medicinal biopolymer was applied to infected mice and gave results with complete recovery and outstanding efficiency.

The exams

Determination of median lethal(LD50)

The average lethal dose was determined in the experiment on 6 mice (three males and 3 females) with using 2% of the drug, mice were monitored for 72 hours and RA was found to have no toxic effect on mice.

Studying the effect of RAD as an antidote on the growth of cancerous tumors
lactobacilli implanted in mice

The method of work

The study of cells cultured in vitro started from implanting a sample of murine spontaneous mammary adenocarcinoma to female mice and for more than 38 passes into the living organism and used as tumor models for animals to study. laboratory animals.

4. Conclusions

The compound RAD showed effective activity as an anti-tumor growth agent, and this was manifested in inhibiting growth and twice as much if the drug was alone at the end of the trial period .

RAD. It is considered one of the advanced antibiotics for the treatment and good inhibition of cancerous tumors, and it has a higher doubling rate than known D because it is linked to the DNA extracted from onions, which is also one of the well-known food anti-cancer agents . RAD compound shows activity in inhibiting cancerous tumors for a longer period, and is thermally stable.

The new compound RAD has high selectivity and can be adopted as a drug for cancerous tumors and the possibility of its use as high development, as it gave double distinguished results without side effects on**.** living cells

Figure-A showing the tumor growth inhibition induced by the anti-tumor agent under study (RAD and D)

Acknowledgement

This work of this research was worked and supplied by Collage of Science, Al-mustansiriyah University, in Department of Chemistry.

References

[1] J. Retuert and M. Yazdani-Pedram, "Cocatalyst effect in potassium persulfate initiated grafting onto chitosan," *Polymer Bulletin*, vol. 31, no. 5, pp. 559-562, 1993.

[2] V. Singh, D. N. Tripathi, A. Tiwari, and R. Sanghi, "Microwave promoted synthesis of chitosangraftpoly (acrylonitrile)," *Journal of Applied Polymer Science*, vol. 95, no. 4, pp. 820-825, 2005.

[3] S.-K. Kim, *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. CRC Press, 2010.
[4] L. Grushevskaya, R. Aliev, and V. Y. Kabanov, "Radiation-induced graft polymerization of acrylamide and acrylic acid onto polyethylene," *International Journal of Radiation Applications and Instrumentation. Part C. Radiation Physics and Chemistry*, vol. 36, no. 3, pp. 475-479, 1990.
[5] V. Singh, A. Tiwari, D. N. Tripathi, and R. Sanghi, "Microwave enhanced synthesis of chitosan-graft-polyacrylamide," *Polymer*, vol. 47, no. 1, pp. 254-260, 2006.
[6] A. Michiels, W. Van den Ende, M. Tucker, L. Van Riet, and A. Van Laere, "Extraction of high-quality genomic DNA from latex-containing plants," *Analytical biochemistry*, vol. 315, no. 1, pp. 85-89, 2003.
[7] Q. Xu, X. Wen, and X. Deng, "A simple protocol for isolating genomic DNA from chestnut rose (Rosa roxburghii Tratt) for RFLP and PCR analyses," *Plant Molecular Biology Reporter*, vol. 22, no. 3, pp. 301-302, 2004.
[8] K. Kathiresan, "Eco-biology of Mangroves," *Mangroves: Ecology, Biology and Taxonomy*, pp. 1-50, 2011.
[9] K. Kathiresan and B. L. Bingham, "Biology of mangroves and mangrove ecosystems," 2001.
[10] W. M. Bandaranayake, "Bioactivities, bioactive compounds and chemical constituents of mangrove plants," *Wetlands ecology and management*, vol. 10, no. 6, pp. 421-452, 2002.
[11] K. Weising, H. Nybom, M. Pfenninger ,K. Wolff, and W. Meyer, *DNA fingerprinting in plants and fungi*. CRC press, 1994.
[12] G. Fang, S. Hammar, and R. Grumet, "A quick and inexpensive method for removing polysaccharides from plant genomic DNA," *Biotechniques*, vol. 13, no. 1, pp. 52-4, 56, 19.92
[13] R. N. Pandey, R. P. Adams, and L. E. Flournoy, "Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides," *Plant Molecular Biology Reporter*, vol. 14, no. 1, pp. 17-22, 1996.
[14] N. Do and R. Adams, "A simple technique for removing plant polysaccharide contaminants from DNA," *BioTechniques*, vol. 10, no. 2, pp. 162, 164, 166-162, 164, 166, 1991.
[15] S. Lal, A. Mahajan, W. Ning Chen, and B. Chowbay, "Pharmacogenetics of target genes across doxorubicin disposition pathway: a review," *Current drug metabolism*, vol. 11, no. 1, pp. 115-128, 2010.
[16] T. Šimůnek, M. Štěrba, O. Popelová, M. Adamcová, R. Hrdina, and V. Geršl, "Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron," *Pharmacological reports*, vol. 61, no. 1, pp. 154-171, 2009.
[17] H. Cortes-Funes and C. Coronado, "Role of anthracyclines in the era of targeted therapy," *Cardiovascular toxicology*, vol. 7, no. 2, pp. 56-60, 2007.
[18] M. Gargouri, A. Sapin, B. Arıca-Yegin, J.-L. Merlin, P. Becuwe, and P. Maincent, "Photochemical internalization for pDNA transfection: Evaluation of poly (d, l-lactide-co-glycolide) and poly (ethylenimine) nanoparticles," *International journal of pharmaceutics*, vol. 403, no. 1-2, pp. 276-284, 2011.
[19] P. Zeng, Y. Xu, C. Zeng, H. Ren, and M. Peng, "Chitosan-modified poly (D, L-lactide-co-glycolide) nanospheres for plasmid DNA delivery and HBV gene-silencing," *International journal of pharmaceutics*, vol. 415, no. 1-2, pp.2011 ,266-259 .
[20] R. Osman, P. L. Kan, G. Awad, N. Mortada, A.-E. El-Shamy, and O. Alpar, "Enhanced properties of discrete pulmonary deoxyribonuclease I (DNaseI) loaded PLGA nanoparticles during encapsulation and activity determination," *International journal of pharmaceutics*, vol. 408, no. 1-2, pp. 257-265, 2011.
[21] K. S. Yadav and K. K. Sawant, "Formulation optimization of etoposide loaded PLGA nanoparticles by double factorial design and their evaluation," *Current drug delivery*, vol. 7, no. 1, pp.2010 ,64-51 .
[22] H. Yamamoto, K. Tahara, and Y. Kawashima, "Nanomedical system for nucleic acid drugs created with the biodegradable nanoparticle platform," *Journal of microencapsulation*, vol. 29, no. 1, pp. 54-62, 2012.