

RESEARCH PAPER

Synthesis and Study of Alginate Hydrogel Doped with Silver Nanoparticles and Natural Compounds of Clove Extract against MCF7 Breast Cancer Cell Lines

Ali Al-Hadidi ^{1*}, Abolfazl Barzegar ², Mohammad Khalaj- Kondori ³

¹ Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran

² Research Center of Biosciences and Biotechnology (RCBB), University of Tabriz, Tabriz, Iran

³ Department Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran

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ABSTRACT

Recently, much attention has been paid to nanoparticles as drug carriers for drug delivery, slow and controlled drug release. Drug nanocarriers improve drug performance and reduce drug side effects. The aim of this study is to synthesize alginate-based hydrogel containing silver nanoparticles and clove extract and the antitumor drug doxorubicin and to investigate the kinetics and thermodynamics of the drug release of doxorubicin and clove extract from it. Hydrogels are hydrophilic polymers with a three-dimensional network containing physical or chemical crosslinks that have high swelling and water absorption capabilities. This feature distinguishes hydrogels from other polymers and introduces them as a biocompatible nanoparticle carrier system. In this regard, silver nanoparticles were first prepared by green synthesis method with thyme extract. Then, in order to evaluate the nature, particle size and understand the morphology of the particles, various methods including electron microscopy (SEM), FTIR, EDAX and XRD) were used. Clove extract was also prepared and then hydrogels containing silver nanoparticles, clove extract and doxorubicin were prepared. The results showed that the alginate hydrogel containing silver nanoparticles and clove extract had a high capacity to load doxorubicin by 95% and slow and continuous release of drugs over a period of two weeks with a release of 95% in the tumor tissue. The effect of pH and temperature was investigated and it showed that at pH=5 and temperature of 45 °C, the drug nanocarrier had the highest release and highest efficiency. The smart hydrogel system was evaluated on MCF7 cancer cells. The IC₅₀ values for hydrogels containing doxorubicin, silver, and clove extract, hydrogel containing clove and silver, hydrogel containing clove and doxorubicin, hydrogel containing silver nanoparticles and doxorubicin, and hydrogels containing all three agents were 4.425, 4.37, 3133, 349.0, 285.2, 270.3 and 2244 µg/mL, respectively.

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* Corresponding Author Email: ali.hu.lafta@utq.edu.iq



INTRODUCTION

Breast cancer is the second most common cancer diagnosed in women, after non-melanoma skin cancers. It is the leading cause of cancer-related death among women worldwide. In the United States, breast cancer is the second leading cause of cancer-related death among women, after lung cancer, with approximately 316,000 new cases diagnosed annually [1,2]. Women at risk of carrying BRCA1/2 mutations or other hereditary cancer susceptibility genes should be evaluated for genetic testing and genetic counseling. However, five-year breast cancer risk assessment tools are not designed to estimate risk in individuals with BRCA1/2 mutations [3]. Ductal carcinoma in situ (DCIS) is classified as stage 0 breast cancer and is considered a noninvasive form of the disease. Early invasive breast cancer is classified as stages I, IIA, and IIB, whereas locally advanced breast cancer is classified as stages IIIA, IIIB, and IIIC. All of these stages are considered non-metastatic breast cancer, whereas stage IV represents metastatic breast cancer [4]. DDSs are technological platforms designed to formulate, protect, and deliver therapeutic agents in appropriate dosage forms, such as tablets, capsules, or injectable formulations. These systems facilitate the delivery of drugs to specific target sites in the body, thereby maximizing therapeutic efficacy while minimizing drug accumulation in non-target tissues [5,6]. In recent years, significant progress has been made in the development of organic-, inorganic-, and hybrid nanoparticle-based drug delivery systems as carriers for active targeted drug delivery, particularly in cancer chemotherapy. Novel DDSs have been designed with improved characteristics, including reduced particle size, enhanced permeability, increased drug solubility, improved therapeutic efficacy, site-specific targeting, greater stability, lower toxicity, and sustained drug release. These properties contribute to markedly improved therapeutic performance compared with conventional drug formulations [7,8]. In recent years, nanoscale drug delivery systems have gained considerable attention. By employing nanocarriers, these systems enable controlled delivery and release of therapeutic agents in the body, creating new opportunities as well as new challenges in the field of nanomedicine [9].

Importantly, AgNPs have been reported to exert stronger antitumor effects in immunocompetent mice than in immunodeficient mice. This

observation suggests that AgNP administration may stimulate antitumor immune responses within the tumor microenvironment [10]. Hydrogels are promising and efficient platforms for the controlled delivery of therapeutic agents in the treatment of various diseases, including diabetes and cancer. Their diverse physicochemical properties enable precise control over drug release while protecting encapsulated drugs from premature degradation [11,12]. Alginate is a naturally occurring anionic polysaccharide primarily extracted from brown seaweeds. Owing to its biocompatibility, low toxicity, relatively low cost, and mild gelation under physiological conditions in the presence of divalent cations such as Ca^{2+} , alginate has been extensively investigated for biomedical applications [13]. The anticancer activity of clove has been extensively investigated in recent years. Numerous studies have demonstrated that clove and its bioactive constituents possess significant anticancer potential. For example, treatment of the thyroid cancer cell line HTh-7 with a clove essential oil nanoemulsion significantly reduced colony formation, indicating potent antiproliferative activity [14].

Anti-tumor activity: The antitumor effects of clove have been demonstrated in both in vitro and in vivo studies. The ethyl acetate fraction of clove extract exhibits significant antiproliferative and antitumor activities [15].

The aim of this study is to synthesize alginate-based hydrogel containing silver nanoparticles and clove extract and the antitumor drug doxorubicin and to investigate the kinetics and thermodynamics of the drug release of doxorubicin and clove extract from it.

MATERIALS AND METHODS

The method of synthesizing silver nanoparticles by green method

At first, 0.085 grams of clove extract was weighed, then poured into Earl Meyer and dipped with 20 ml of dionis water. With one to one to one 0.085 grams of silver nitrate in 10 ml of dionis water, then the drop was added to the clove extract and placed on the magnetic strategy for 40 minutes. The discoloration from yellow to dark brown was a sign of the revival of silver salts to the silver nanoparticles. Then the sediment was washed once and dried inside the oven for 5 hours at 70 °C. Silver nanoparticles were examined using XRD, FTIR, DLS and SEM. For the synthesis

of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of dionice water and then used with 4 % calcium chloride solution. The sample was then frozen and dried with a freeze-dryer after 24 hours. Hydrogels prepared by XRD, FTIR and SEM tests were investigated.

Preparation of Dox/Alginat hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4 % hydrogel calcium chloride. Hydrogels prepared by XRD, FTIR and SEM tests were investigated.

Preparation of ag/alginat hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4 % hydrogel calcium chloride. Hydrogels prepared by XRD, FTIR and SEM tests were investigated.

Preparation of clove/alginat hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4 % hydrogel calcium chloride. Hydrogels prepared by XRD, FTIR and SEM tests were investigated.

Preparation of ag/alginat/dox hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4 % hydrogel calcium chloride. Hydrogels prepared by XRD, FTIR and SEM tests were investigated.

Preparation of ag/alginat/clove hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4 % hydrogel calcium chloride.

Hydrogels prepared by XRD, FTIR and SEM tests

Preparation of Dox/ALGINAT/clove hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4 %

hydrogel calcium chloride.

Hydrogels prepared by XRD, FTIR and SEM tests DOX /ALGINAT /AG /Clove Hydrogels

For the synthesis of hydrogel alginate, 1.5 % volume of alginate was dissolved in 40 ml of deionized water. The doxorubicin was then added to the soluble bed and then prepared with 4% hydrogel calcium chloride.

Hydrogels prepared by XRD, FTIR and SEM tests Investigation of the in vitro release of clove extract from alginate hydrogel

The following method was used to release clove extract from alginate hydrogel loaded with clove extract. In this way, 0.015 g of drug-containing nanoparticles (after removing the unloaded drug) in 3 ml of phosphate buffered saline (PBS) solution was investigated under two conditions (37 °C, pH=7.4) and (40 °C, pH=3.5), which are the external physiological stimulus conditions and cancer tissue conditions, respectively. This process was carried out by sampling the external solution at specific time intervals and determining the Clove concentrations in the sampled solution with a UV-Vis spectrophotometer. At each sampling step, an equal volume of phosphate buffered saline solution was added to the system to replace the volume of the sampled solution. The percentage of Clove drug released was calculated as a percentage of the total drug present in the nanoparticle structure using the Clove calibration curve and plotted as a function of time.

Investigation of the in vitro release of silver nanoparticles from alginate hydrogel

In order to release silver nanoparticles from silver-loaded alginate hydrogel, the following method was used. In this way, 0.015 g of drug-containing nanoparticles (after removing the unloaded drug) were dissolved in 3 ml of phosphate buffered saline (PBS) solution under two conditions (37 °C, pH=7.4) and (40 °C, pH=3.5), which are the external physiological stimulus conditions and cancer tissue conditions, respectively. This process was carried out by sampling the external solution at specific time intervals and determining the Ag concentrations in the sampled solution with a UV-Vis spectrophotometer. At each sampling stage, an equal volume of phosphate buffered saline solution was added to the system to replace the volume of the sampled solution. The percentage

of Ag drug released was calculated as a percentage of the total drug present in the nanoparticle structure using the Ag calibration curve and plotted as a function of time.

Toxicity study of hydrogel loaded with doxorubicin, clove extract and silver nanoparticles in vitro

Toxicity study to evaluate the anticancer effect of loaded hydrogels with doxorubicin, clove extract and silver nanoparticles was carried out in vitro through the MTT test. First, colon cancer MCF7 cells were cultured as a monolayer in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) in the presence of 5% CO₂. Then, they were placed in an incubator at 37°C. After reaching 80% of the flask surface (Confluency), the cells were cultured in 96-well plates. To evaluate the toxicity of the drug on these cells, they were exposed to different groups and dilutions of soluble doxorubicin, nanoparticles, clove extract, hydrogels containing the drug and hydrogels containing no drug and containing clove extract and silver nanoparticles for 24, 48 and 72 hours, and the toxicity was measured using the MTT test. Hydrogels containing the drug and silver nanoparticles and clove extract and without it and free drug were used with different concentrations. After the mentioned treatment times, the culture medium was discarded along with the different groups. It was washed once with phosphate buffer saline. Then, 100 microliters of culture medium and 100 microliters of MTT solution (with a concentration

of 5 mg/ml) were added to each well of 96-well plates and incubated for 3 hours in the dark in an incubator at 37°C. After this period, the above solution was withdrawn and 200 microliters of dimethyl sulfoxide (DMSO) was added to each well. Then, their absorbance was read at 570 nm by ELISA reader and the percentage of viable cells was evaluated by comparing the control (untreated grown cells with any of the groups).

RESULTS AND DISCUSSION

Examination of the results obtained from XRD

To examine the structure of Ag nanoparticles, doxorubicin and alginate hydrogels, X-ray diffraction (XRD) was used in the region of 20 to 90 in terms of θ . Figs. 1 shows the XRD spectrum diagram of the above-mentioned samples. Fig. 1 is the diagram of the Ag nanoparticles sample. As can be seen in the figure, the peaks of silver nanoparticles are seen at θ values of 1.38, 3.44, 5.64, 7.77 and 5.82 with Miller indices (111), (200), (220), (311) and (331) respectively. The peaks correspond to the XRD pattern of silver nanoparticles.^[1]

Review of the results obtained from FTIR

To determine the type of synthesized materials and hydrogels containing Ag, doxorubicin drug, and clove extract, to obtain the functional groups present in their structure, and also to prove the successful encapsulation of Ag nanoparticles, clove extract, and doxorubicin, the FTIR spectrum of the prepared samples was taken in the wave

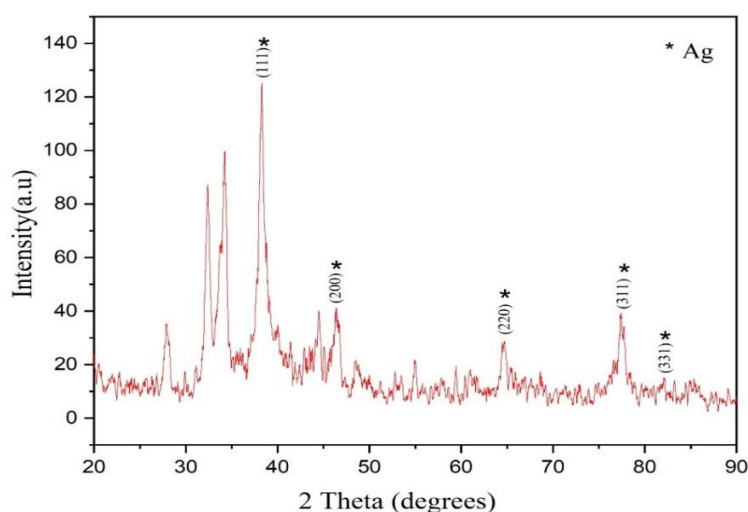


Fig. 1. X-Ray diffraction Pattern XRD

number range of 400 cm^{-1} to 4000 cm^{-1} .

FTIR spectrum of silver nanoparticles

Fig. 2 shows the FTIR spectrum of silver nanoparticles. The absorption bands centered at 1080, 1385, 1629, 3389 and 1080 are associated with the C-O stretching vibration and are assigned to the ester bonds. The peak at 1629 is related to amide I, resulting from the carbonyl stretching vibrations, the peak at 3389 refers to the stretching vibration of primary amines.

FTIR spectrum of anticancer drug doxorubicin

Fig. 3 shows the FTIR spectrum of doxorubicin. As in Figs. 3, which is related to doxorubicin, a peak is seen at 1011 cm^{-1} , which is related to C-O vibrations. Also, several peaks are observed in the doxorubicin spectrum in the regions of 1718, 1618.51, 1584.53, 1439, and 1297 cm^{-1} , which can be related to (C=O), (N-H), (C-C), and (C-H) vibrations, respectively.

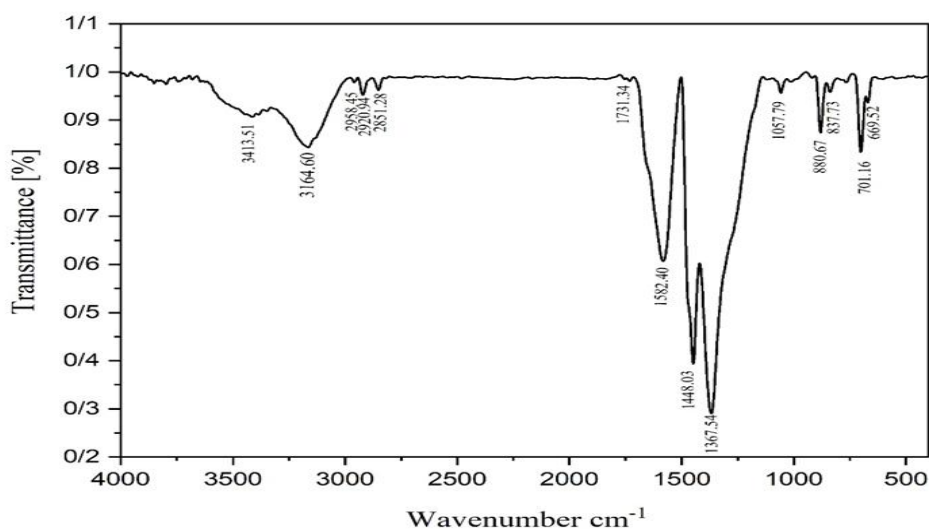


Fig. 2. FTIR spectrum.

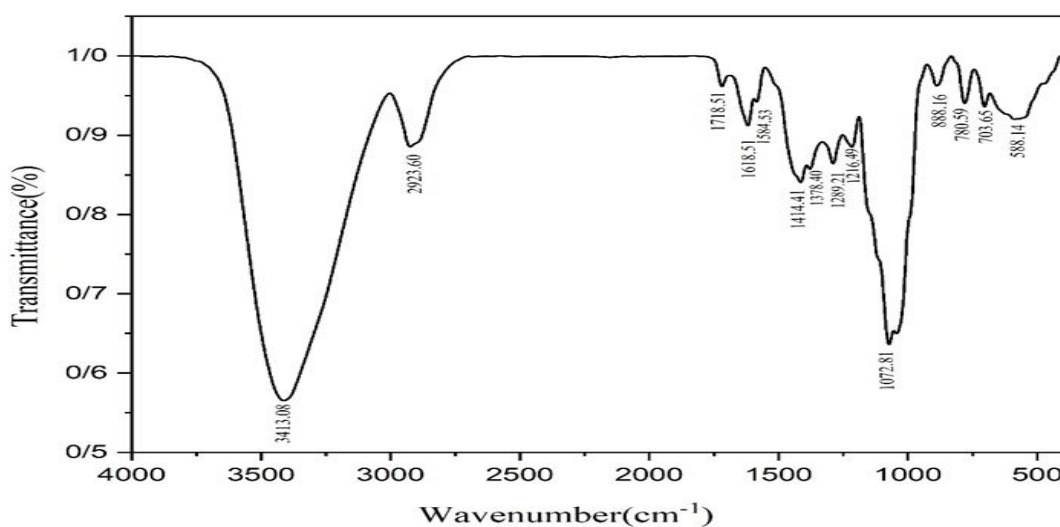


Fig. 3. IR spectrum.

FTIR spectrum of sodium alginate

FTIR spectroscopy was used to characterize sodium alginate by identifying functional groups and analyzing its molecular structure. The key peaks in the FTIR spectrum of sodium alginate include peaks at 3410 cm^{-1} , 1635 cm^{-1} , 1419 cm^{-1} and 1050 cm^{-1} , which are related to the stretching vibrations of hydroxyl, asymmetric COO, symmetric COO and C-O, respectively (Fig. 4).

FTIR spectrum of alginate hydrogel

FTIR spectrum was used to investigate sodium alginate hydrogel by identifying functional groups

and analyzing its molecular structure. The key peaks observed in the FTIR spectrum of sodium alginate were also observed in this spectrum for alginate hydrogels, including peaks at 3410 cm^{-1} , 1635 cm^{-1} , 1419 cm^{-1} and 1050 cm^{-1} , which are related to hydroxyl, asymmetric COO, symmetric COO and C-O stretching vibrations, respectively (Fig. 5).

Review of the results obtained from FE-SEM, EDX and MAP images

The size and surface morphology of silver nanoparticles and synthesized alginate hydrogels

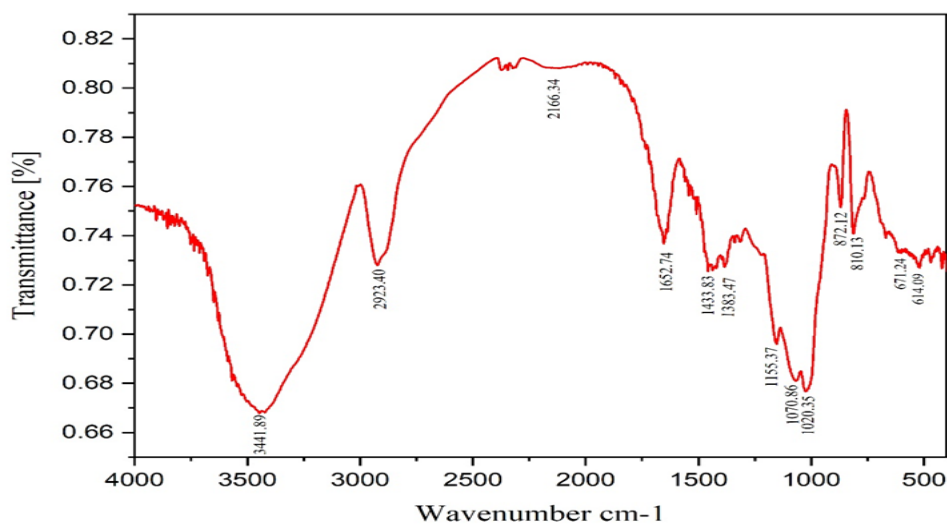


Fig. 4 FTIR Fourier transform infrared spectroscopy.

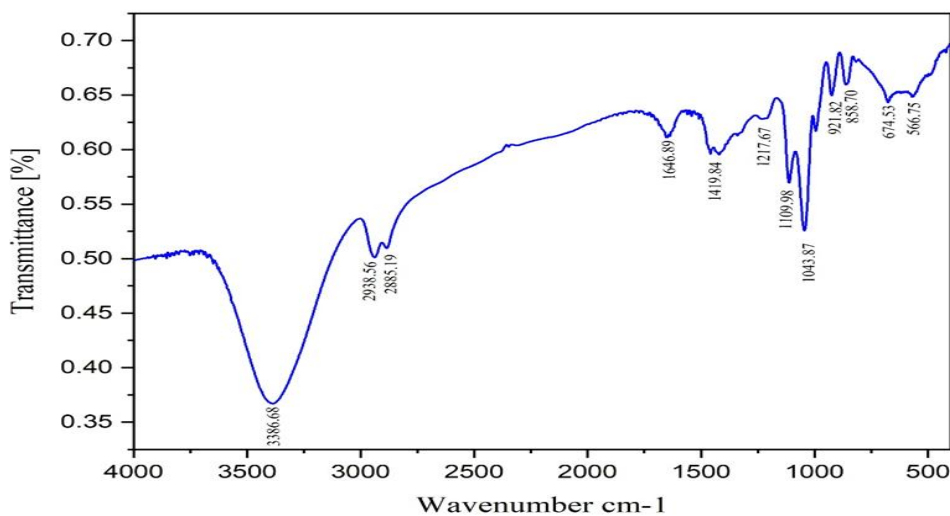


Fig. 5. IR spectrum.

were examined by field emission scanning electron microscopy (FE-SEM). The images of silver nanoparticles are given Fig. 6.

Review of morphology and elemental analysis of silver nanoparticles

Fig. 6 shows the microscopic images of silver nanoparticles. As it is clear, nanoparticles synthesized by the green method were observed. In the green synthesis, the morphology of nanoparticles was obtained as complex and

spherical with a size distribution of 32.5 nm. Fig. 8 and Table 1 shows the elemental analysis diagram of silver nanoparticles. The presence of elements indicates the presence of silver nanoparticles. In the diagram related to the green synthesis of nanoparticles, in addition to the mentioned elements, C and N are also present.

Morphology and Elemental Analysis of Alginate Hydrogels

Fig. 9 shows microscopic images of alginate

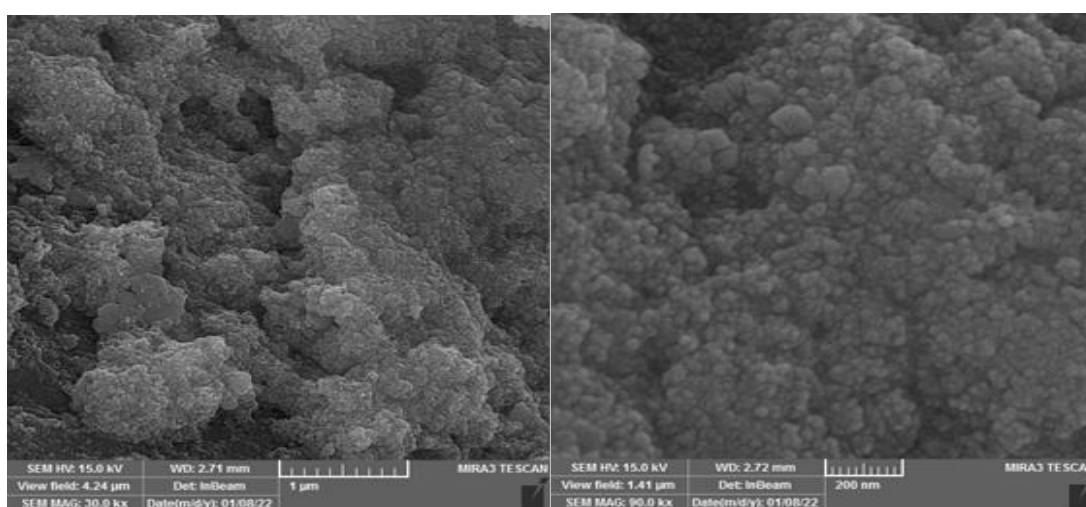


Fig. 6. Field emission electron microscope images of synthesized samples - silver nanoparticles by the green method.

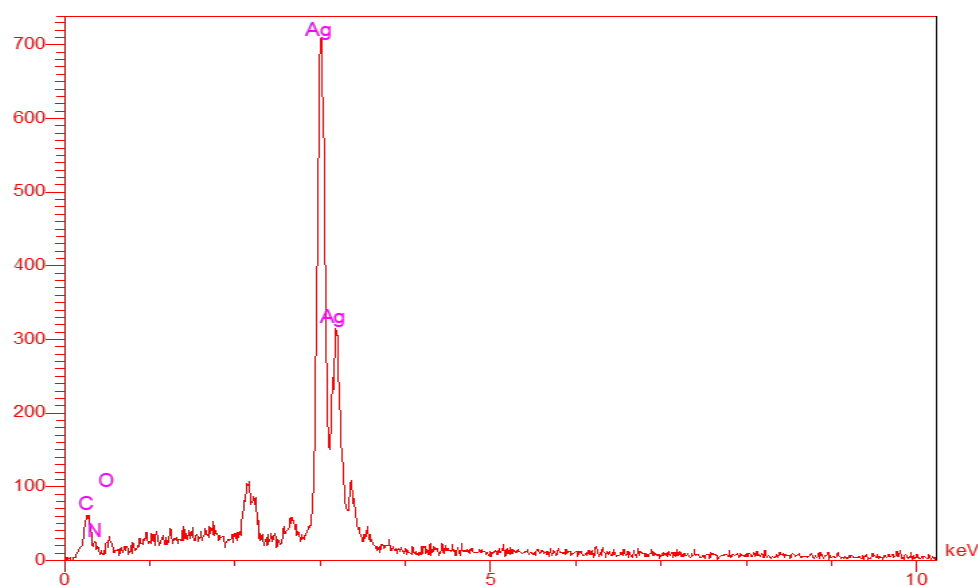


Fig. 7. EDX graph a silver nanoparticles synthesized by green method.

hydrogels. As can be seen, hydrogels have a porous structure for the release of encapsulated agents.

Morphology and elemental analysis of alginate hydrogel containing silver nanoparticles, clove extract, and the anticancer drug doxorubicin as can be seen in the images of hydrogels encapsulated

with nanoparticles, doxorubicin, and clove extract, the morphology of hydrogels containing different factors is different from empty hydrogels. Fig. 11 shows the elemental analysis diagram of hydrogels containing doxorubicin, silver nanoparticles, and clove extract. The presence of a high percentage of

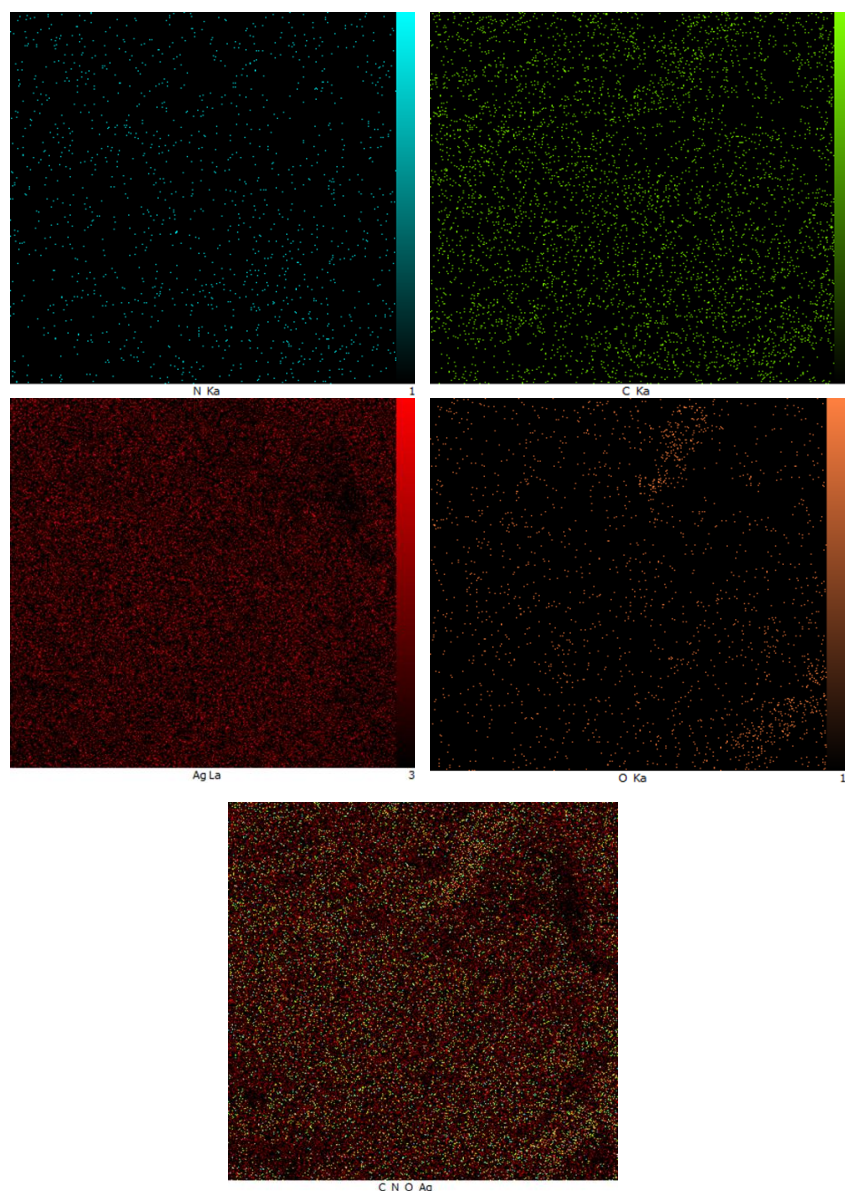


Fig. 8. MAP analysis of silver nanoparticles synthesized by green synthesis method.

Table 1. EDX analysis of Ag nanoparticles by chemical and green synthesis methods.

Sample	C	O	N	Ag
Green synthesis Ag	9.91	2.95	2.46	8469

O, C elements indicate the loading of doxorubicin, and a high percentage of silver atoms and a high percentage of O, C elements are related to the eugenol compound, which is a compound with antimicrobial, anticancer, antioxidant, anti-inflammatory, and analgesic properties, inside the hydrogel.

In vitro cytotoxicity study of doxorubicin-loaded hydrogels

Biocompatibility is a very important factor in

the properties of nanocarriers for modern drug delivery systems. MTT analysis was performed to investigate the viability of the cell (MCF7 cancer cell line) and to investigate the therapeutic effect of doxorubicin-loaded hydrogels. The Figs. 13-15 show the toxicity effects for 1) free drug, 2) drug-free hydrogels, 3) drug-containing hydrogels, 4) hydrogels with silver nanoparticles, 5) hydrogels containing clove extract, 6) hydrogels containing silver and doxorubicin, 7) hydrogels containing silver with cloves, 8) hydrogels

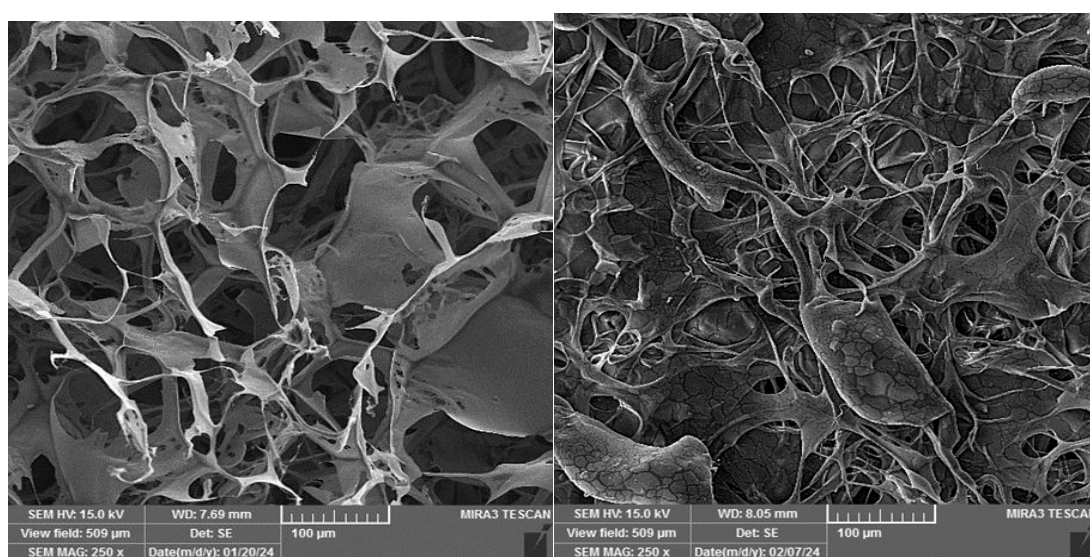


Fig. 9. Field emission electron microscope images of alginate hydrogels.

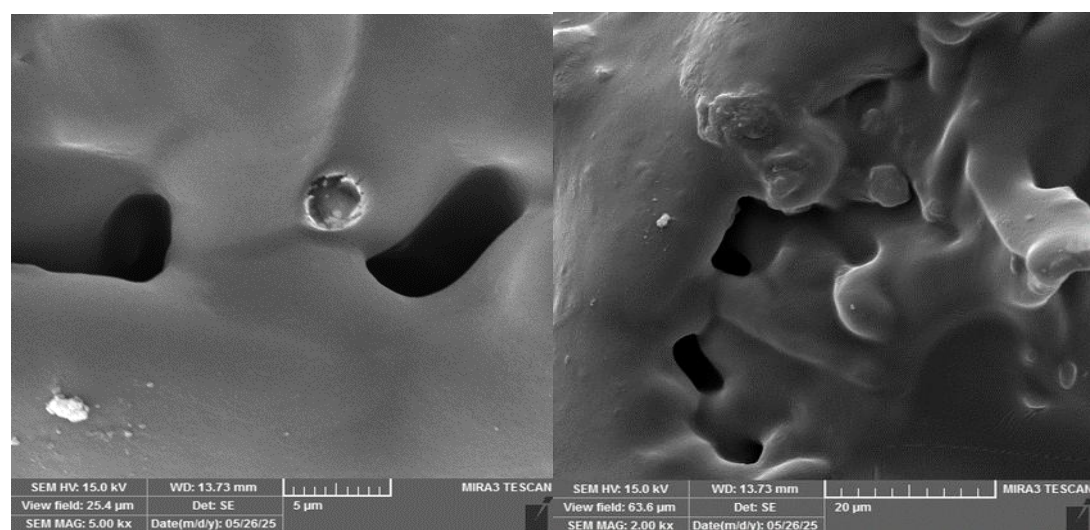


Fig. 10. SEM scanning electron microscope image.

containing doxorubicin and cloves, and 9) hydrogels containing all three samples at different concentrations at times of 24, 48, and 72. It can be seen that after 72 hours of incubation of cells with hydrogels containing silver nanoparticles and clove extract and doxorubicin, they had significant toxicity on the viability of cancer cells. As is clear from the figures, the free drug had more toxicity on the cells and growth inhibition on them than the other groups. In the comparison between the groups of silver nanoparticles and hydrogels containing nanoparticles and drug and clove extract, the functional role of hydrogels as acceptable carriers for drug delivery systems for slow and continuous release of drugs can be understood. In the comparison between the nanocomposites without and containing drug, it can also be concluded that the nanocomposite without drug does not have significant cytotoxicity at the highest concentration used. In the comparison between the hydrogel containing drug and the hydrogel containing silver nanoparticles and clove extract, it can be understood that the hydrogel containing drug has a significant toxicity compared to the other two groups. Finally, the

results showed that the designed hydrogels have high potential as targeted drug delivery systems. The IC₅₀ values were calculated for the groups. The IC₅₀ values were 4.425, 40.37, 313.3, 349.0, 285.2, 270.3, and 2.244 µg/ml for the hydrogels containing doxorubicin, silver, and clove extract, the hydrogel containing clove and silver, the hydrogel containing clove and doxorubicin, the hydrogel containing silver nanoparticles and doxorubicin, and the hydrogels containing all three agents, respectively.

Breast cancer (BC) remains one of the most prevalent and life-threatening malignancies worldwide, with millions of new cases diagnosed annually. According to the GLOBOCAN 2021 report, BC accounted for 11.7% of all cancer cases, making it the most common cancer globally, followed by lung cancer (11.4%) and colorectal cancer (10.0%) [16]. Despite progress in diagnostic methods and therapeutic interventions, chemotherapy remains the cornerstone of treatment. However, its effectiveness is often limited by severe side effects, drug resistance, and lack of specificity toward tumor tissues, which can result in significant harm to normal cells. Consequently, there is an

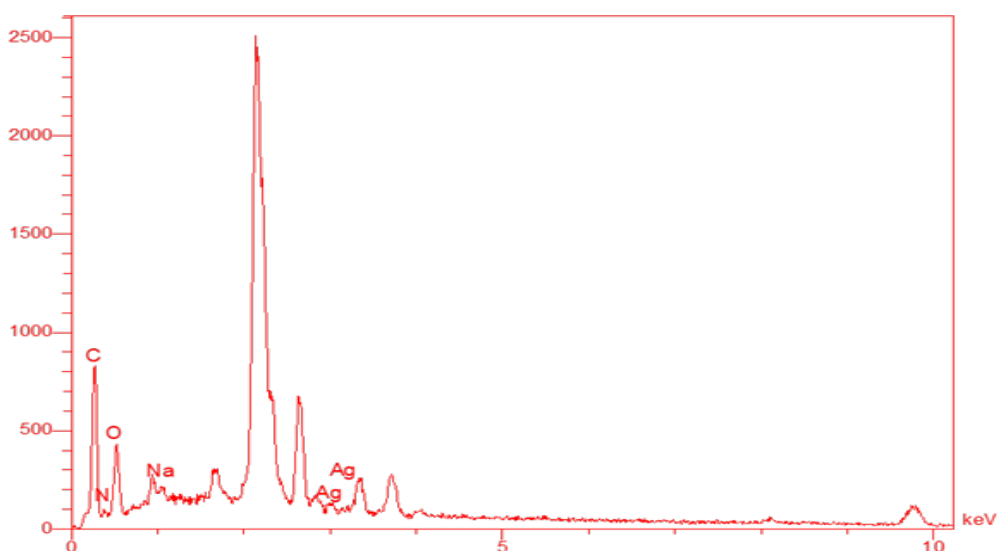


Fig. 11. EDS spectrum.

Table 2. EDX analysis of hydrogels containing doxorubicin, clove extract and silver nanoparticles.

Sample	Na(wt%)	O(wt%)	C(wt%)	Ag(wt%)
Ag-Clove-Dox@Alginat	0.18	31.82	51.86	14.63

urgent need to develop novel, targeted, and biocompatible drug delivery systems to overcome the limitations of conventional chemotherapy.

Nanoparticle-based drug delivery systems (NDDS) have emerged as promising platforms due to their unique physicochemical properties. Their nanoscale size (<100 nm), high surface-to-volume ratio, and tunable morphology enable

enhanced solubility, stability, and bioavailability of encapsulated drugs [17]. Moreover, the enhanced permeability and retention (EPR) effect promotes preferential accumulation of nanoparticles at tumor sites, thereby improving therapeutic efficacy while reducing systemic toxicity. Among various carriers, alginate-based hydrogels are particularly attractive because of their high drug-loading

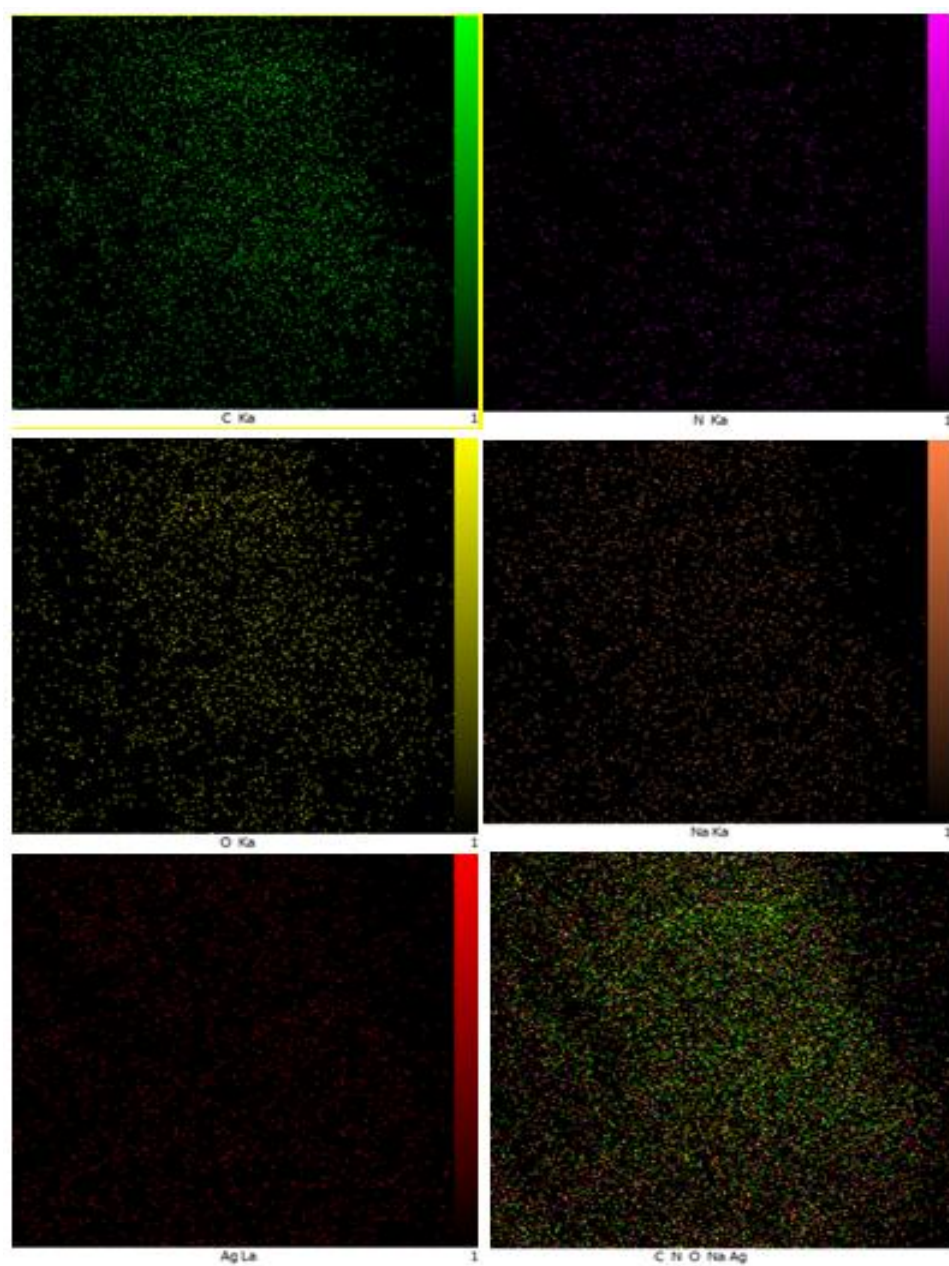


Fig. 12. Elemental mapping.

capacity, biocompatibility, biodegradability, and ability to provide sustained and pH-responsive drug release [18].

In this study, a rapidly developed alginate hydrogel system containing silver nanoparticles, clove extract, and the drug doxorubicin was used to evaluate its anticancer efficacy against MCF7 cancer cells. He added that all three agents, whether tested individually or in binary combinations, exhibited dose- and time-dependent cytotoxic effects, and our study's results were consistent with those of a previous study[19]. Importantly, the triple combination encapsulated within the hydrogel exhibited the

strongest cytotoxicity, with the lowest IC₅₀ values compared to single or dual formulations [20]. This observation confirms the synergistic interaction between natural and synthetic agents, thereby enhancing the overall therapeutic potential of the hydrogel system [21].

The mechanisms underlying this synergistic effect can be attributed to the distinct yet complementary activities of the incorporated agents. Silver nanoparticles are known to induce apoptosis in tumor cells primarily through oxidative stress and the generation of reactive oxygen species [22]. Clove extract, particularly its major phenolic compound eugenol, has been reported

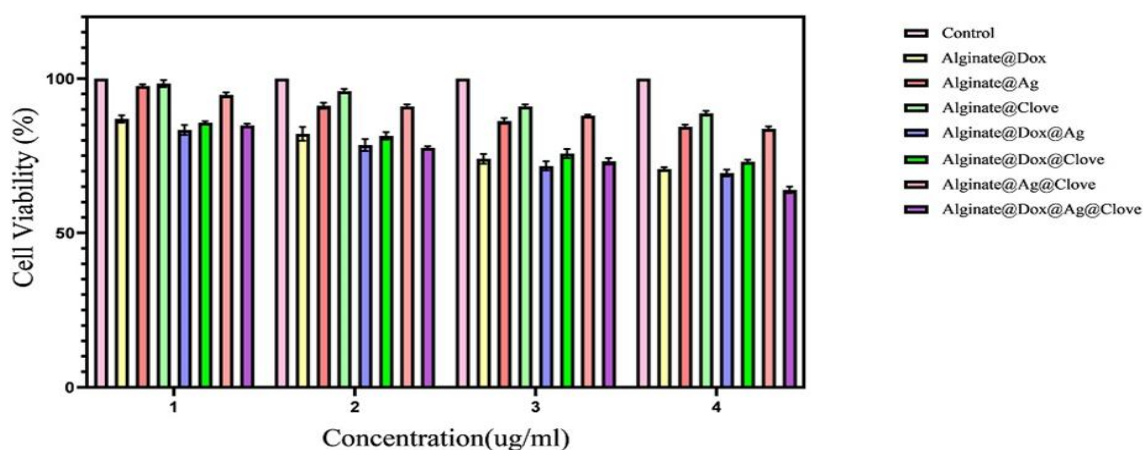


Fig. 13. MCF7 cell viability after incubation with free drug and hydrogels loaded with doxorubicin, silver nanoparticles, and clove extract for 24 hours.

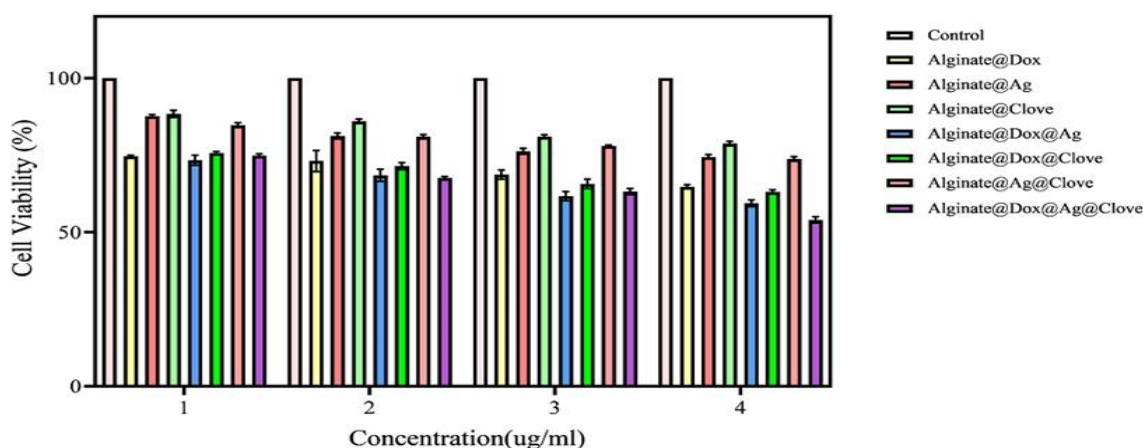


Fig. 14. MCF 7 cell viability after incubation with free drug and hydrogels loaded with doxorubicin, silver nanoparticles, and clove extract for 84 hours.

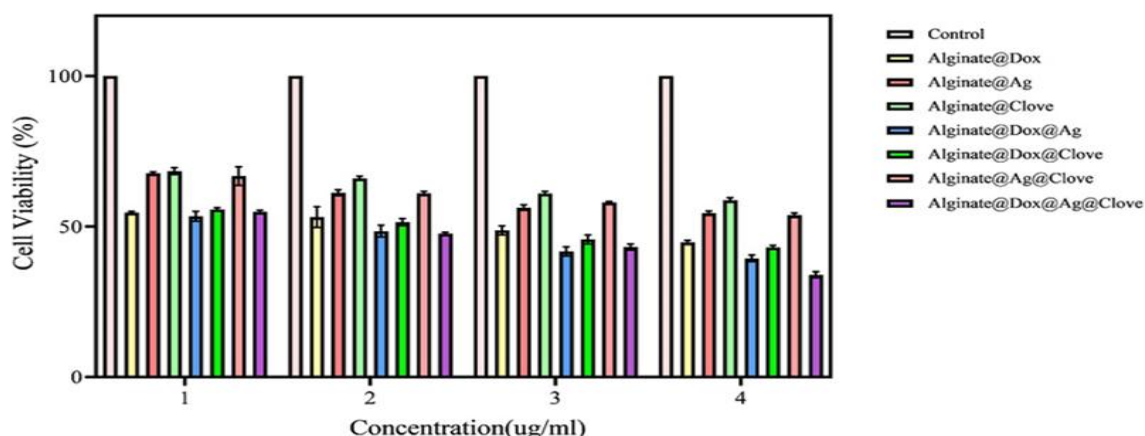


Fig. 15. MCF 7 cell viability after incubation with free drug and hydrogels loaded with doxorubicin, silver nanoparticles, and clove extract for 72 hours.

to exert antioxidant, anti-inflammatory, and antiproliferative activities by modulating signaling pathways associated with cell cycle progression and apoptosis. When combined with doxorubicin, which functions through DNA intercalation and inhibition of topoisomerase II, these agents act synergistically to enhance cytotoxicity against cancer cells while potentially reducing the effective dose required for doxorubicin [23].

Another key finding of this research was the ability of the alginate hydrogel to provide controlled and sustained drug release. The release profile revealed that the hydrogel exhibited optimal performance under acidic conditions (pH = 5) and elevated temperature (45 °C), conditions that closely resemble the tumor microenvironment. This property allowed for gradual release of therapeutic agents, leading to prolonged cytotoxic activity, increased drug accumulation at the tumor site, and reduced systemic toxicity. These findings highlight the promise of smart hydrogel systems as targeted and efficient drug delivery platforms for cancer therapy [24].

Despite the encouraging outcomes, several limitations of this study must be acknowledged. First, the experiments were conducted exclusively under *in vitro* conditions, which may not fully replicate the complexity of the tumor microenvironment *in vivo*. Therefore, further validation using animal models is necessary to confirm the therapeutic efficacy, biodistribution, and safety of the developed hydrogel system. Second, detailed mechanistic investigations are

required to elucidate the molecular pathways modulated by the combined action of silver nanoparticles, clove extract, and doxorubicin. Such insights could inform the rational design of future hydrogel formulations with optimized properties. Finally, long-term studies, including pharmacokinetic and toxicological evaluations, are needed to assess the translational potential of this nanohydrogel system in clinical practice.

CONCLUSION

Overall, this study demonstrated that alginate hydrogel loaded with silver nanoparticles, clove extract, and doxorubicin represents a highly promising multifunctional drug delivery system with potent cytotoxic effects against MCF7 breast cancer cells. By integrating the complementary mechanisms of synthetic and natural agents within a biocompatible and pH-responsive hydrogel matrix, the system not only enhanced therapeutic efficacy but also addressed some of the major drawbacks of conventional chemotherapy. While further *in vivo* and clinical investigations are essential, the findings of this research provide a solid foundation for the development of next-generation smart nanohydrogel systems for targeted breast cancer therapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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