Jordan Journal of Pharmaceutical Sciences, Volume 18, No. 4, 2025

Formulation, characterization, and cytotoxicity evaluation of doxorubicin loaded niosomes prepared by microfluidic mixing

Mohammad A. Obeid[1*], Suojoud AL-tbinat[2], Haneen Amawi[3], Alaa A. A. Aljabali[1], Bahaa Altrad[2]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Yarmouk University, Irbid, Jordan.
[2] Department of Biological Science, Faculty of Science, Yarmouk University, Irbid, Jordan
[3] Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Yarmouk University, Irbid, Jordan

ABSTRACT

Doxorubicin (DOX) is one of the common anticancer agents used for treating various types of cancer. One limitation of DOX is the development of cancer cell resistance and its wide range of toxicity. Niosomes are type of nanoparticles that consist of non-surfactants and cholesterol in the form of a bilayer structure. The aim of the present study was to develop and optimize DOX-loaded niosomes using microfluidic mixing through buffer exchange strategy to overcome DOX limitations such as unwanted distribution and toxicity. Two niosome formulations (F1 and F2) were prepared using T85 as a non-ionic surfactant. F1 was composed of TW85: CHOL (40:60)/DOX, while F2 was composed of TW85: CHOL: DDAB (40:40:20)/DOX. F1 had an anionic surface charge of around -13 mV, while F2 had a cationic charge of around +20 mV. DOX was successfully entrapped in both formulations with entrapment efficiencies of approximately 11% for F1 and 79% for F2, respectively. Loading DOX into F1 resulted in a significant reduction in the IC50 value in both MCF7 and A549 cells. However, loading DOX into F2 resulted in a higher IC50 in both cell lines compared to the free DOX. In conclusion, the optimized DOX niosomes formulation F1 improved the anticancer activity of DOX compared to free DOX drug. These results demonstrate that optimized niosomes can be effective tools for delivering DOX to target breast and lung cancer cells.

Keywords: Niosomes, Doxorubicin, microfluidic mixing, nanotechnology, drug delivery.

INTRODUCTION

Cancer arises due to the irregular and uncontrolled growth of abnormal cells, and these malignant cells have the ability to spread in the affected organ and in other parts of the body [1]. Cancer is a global health problem and is considered the second leading cause of death in the United States of America [2]. With around 10 million new cases annually, the World Health Organization estimates cancer-related deaths to reach around 13.1 million by 2030 [1]. Many strategies have been developed for cancer treatment, such as chemotherapy, which utilizes various anti-cancer drugs including Docetaxel, Bleomycin, Fludarabine, Hydroxyurea, Cisplatin, and Doxorubicin (DOX).

Doxorubicin (DOX) has been used for a long time to treat tumors [3]. It is effective against various types of malignant solid ~~cancers~~ and hematologic cancers [4]. Cell death occurs due to the strong interaction of DOX with cellular nuclei and its intercalation with DNA base pairs, forming anthracycline-DNA complexes [5]. This interaction halts the transcription process and inhibits RNA and DNA synthesis. DOX also inhibits the enzyme topoisomerase II, which leads to DNA damage and induces cell apoptosis through oxidative stress caused by

[*]***Corresponding author: Mohammad A. Obeid***
m.obeid@yu.edu.jo
Received: 24/08/2024 Accepted: 03/11/2024.
DOI: https://doi.org/10.35516/jjps.v18i4.3218

free radicals [6].

Unwanted and harmful side effects of DOX include cardiotoxicity, hepatotoxicity, myelosuppression, vomiting, and diarrhea [7]. Additionally, chemotherapy failures can occur due to the development of multidrug resistance associated with DOX use. Consequently, delivery systems have emerged to enhance DOX effectiveness, overcome cancer cell resistance, and improve targeted delivery to reduce side effects [8].

A drug delivery system is a technique used to deliver drugs to specific cells and organs to achieve desired therapeutic outcomes [9]. Nanoparticles (NPs) serve as effective drug carriers due to their small size (1-1000 nm). NPs offer promising prospects in cancer treatment by overcoming drug resistance, reducing toxicity, and enhancing drug efficacy [10, 11]. Various types of NP systems exist, including vesicle carriers like liposomes and niosomes, and both types play crucial roles in drug delivery [12, 13]. Niosomes, composed of non-ionic surfactants and cholesterol in bilayer vesicles, can encapsulate both lipophilic and hydrophilic drugs [14]. Compared to liposomes, niosomes are advantageous due to their low production cost, high stability, ease of manufacture, biodegradability, and non-immunogenicity [15] [16].

Currently, several liposomal DOX formulations are available under brand names such as Doxil®, CaelyxTM, Myocet®, and Zolsketil® [17]. Despite their availability, these formulations suffer from limitations related to the physicochemical stability of liposomes, which are influenced by the stability of their phospholipid constituents. Phospholipids forming liposomes are prone to degradation reactions such as oxidation and hydrolysis necessitating special formulation and storage conditions like preparation using lyophilization technique and storage under low temperatures.[18, 19]. Moreover, liposomal formulations are typically expensive due to the high cost of phospholipids and the need for specialized preparation techniques and storage [20].

Niosomes, similar to liposomes, are vesicular delivery systems consisting of a membrane bilayer encapsulating an aqueous core. However, niosomes are composed of non-ionic surfactants known for their high stability and low production cost [19].

Other types of nanocarriers have been employed for DOX loading and delivery. Kayani et al. prepared β-lactoglobulin NPs integrated with folic acid and loaded with DOX to enhance DOX efficacy against breast cancer cells. β-lactoglobulin NPs achieved a DOX encapsulation efficiency (EE) of approximately 68.82% ± 1.76% and demonstrated significant inhibition of cancer cell growth compared to free DOX [21]. In another study by Verma et al., pegylated liposomal DOX formulations were developed, resulting in improved efficacy and a significantly reduced risk of cardiotoxicity compared to free DOX, whether used alone or in combination with trastuzumab [22, 23].

This study aims to explore the possibility of loading DOX into niosome nanoparticles to develop cost-effective and stable vesicular formulations of DOX. Niosomes were prepared using microfluidic mixing for DOX encapsulation, and the prepared niosomes were evaluated for their physicochemical properties, DOX encapsulation efficiency (EE%), and their cytotoxicity against MCF7 breast cancer cell lines and A549 lung cancer cell lines.

MATERIALS AND METHOD

Materials:

Polyoxyethylene sorbitan trioleate (TW85), cholesterol (Chol), didodecyldimethylammonium bromide (DDAB), ethanol, phosphate-buffered saline (PBS) tablets (pH 7.4), and ammonium sulfate (pH 4.5) were purchased from Sigma Aldrich, UK. DOX was purchased from TCI Chemicals, Japan. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and trypsin EDTA were purchased from Hiclon, Europe. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-

tetrazolium bromide (MTT) powder was purchased from TCI Chemicals, Japan. Dimethyl sulfoxide (DMSO) was purchased from GHTECH, China. The lung cancer cell line (A459) and breast cancer cell line (MCF7) were obtained from the American Type Culture Collection (ATCC) and gifted by Dr. Walhan Alshaer, University of Jordan.

Preparation of blank niosome

Niosome formulations comprising of TW85 and CHOL with or without DDAB were prepared using the microfluidic mixing technique with a pH gradient to enhance drug loading, as previously described in the litrature [24]. F1 niosomes are composed of TW85:CHOL in a 60:40 molar ratio and F2 niosomes are composed of TW85:CHOL: DDAB in a 40:40:20 molar ratio.

Each component of the niosomes was prepared separately as a stock solution. These components were then mixed together at the desired molar ratio to form the lipid phase for the microfluidic system. The prepared lipid phases were mixed with 250 mM ammonium sulfate at pH 4.5 as the aqueous phase using microfluidic chips with dual inlets obtained from Precision NanoSystems, Canada. Both phases were mixed at a flow rate ratio (FRR) of 3:1 (aqueous:lipid) and a total flow rate (TFR) of 8 mL/min. These mixing parameters were achieved by passing the two phases through the microfluidic chips using non-peristaltic syringe pumps obtained from VWR, USA.

Mixing of the two phases through the microfluidic chip resulted in ~~the~~ self-assembly of the lipid components into a bilayer structure with an aqueous core containing 250 mM ammonium sulfate at pH 4.5. To create niosomes with a pH gradient, the prepared niosomes were dialyzed against 10X volume of PBS at pH 7.4 using a dialysis bag (molecular weight cut-off 14 kDa) to replace the external ammonium sulfate with PBS. This process resulted in niosomes with an acidic aqueous core and a neutral external pH [25, 26].

DOX loading into niosomes

For loading DOX into niosomes, a stock solution of DOX was prepared and mixed with the two niosome formulations (F1 and F2) to achieve a final DOX concentration of 0.25 mg/mL, which is equivalent to 10% of the total lipid concentration (2.5 mg/ml). The formulations were then incubated in a water bath at 60°C for one hour to facilitate the diffusion of the drug inside the niosomes.

Niosomes charactarisation

The average particle size, polydispersity index (PDI), and zeta potential (ZP) of niosomes with and without DOX were measured using Zetasizer Nano ZSP system (Malvern Instruments) at 24°C.

Niosomes morphology

Morphological examination of both blank niosome formulations F1 and F2 was conducted using transmission electron microscopy (TEM) with a JEOL JEM-1200EX TEM (JEOL, Tokyo, Japan) operating at an accelerating voltage of 80 kV. For sample preparation, carbon-coated copper grids (400 mesh, Agar Scientific) were glow discharged in air for 30 seconds. Three microliters of samples were drop-cast onto the grids and negatively stained using uranyl acetate. Subsequently, the samples were allowed to dry in a dust-free environment before TEM imaging.

Doxorubicin Encapsulation Efficiency (EE%)

After incubating niosomes with DOX, unencapsulated DOX was removed by dialysis against 10X volume of PBS at room temperature under continuous stirring. Niosomes were then lysed with an equal volume of methanol to release the encapsulated DOX for quantification.

The absorbance of DOX was measured using a microplate reader at a wavelength of 480 nm. The concentration of DOX was determined by constructing a standard curve using known concentrations of DOX and measuring their absorbance at 480 nm using PBS as a blank.

The encapsulation efficiency (EE%) of DOX was calculated using the following equation:

EE % = Encapsulated DOX/Total DOX ×100

Where total DOX is the total amount of DOX added during niosome preparation and encapsulated DOX is the amount of DOX that were encapsulated into niosomes.

Anticancer activity of DOX loaded niosomes

The anticancer activity of DOX-loaded niosomes was evaluated on human breast cancer cell lines (MCF-7) and human lung cancer cell lines (A549). The cancer cell lines were seeded in 96-well plates at a concentration of 5000 cells/well in RPMI media supplemented with 10% FBS and 1% Pen/Strep, and incubated at 37°C, 5% CO2, and 100% humidity. F1 and F2 niosomes, both as blank and DOX-loaded formulations, were applied to each cell line, and the plates were further incubated at 37°C and 5% CO_2 for 24 hours.

Cytotoxicity was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which relies on the conversion of MTT to formazan crystals by mitochondrial reductases in viable cells [27]. This transformation results in a color change that correlates with the percentage of living cells. Twenty µL of MTT solution was added to each well of the treatment plate, and the plates were returned to the incubator for 4 hours at 37°C. Subsequently, the media was carefully removed, and the formazan crystals were dissolved in 180 µL of dimethyl sulfoxide (DMSO). Absorbance was measured using a plate reader at a wavelength of 570 nm, and the IC50 values of each formulation were calculated.

Results and discussions

Recent advancements in nanotechnology drug delivery have enabled the specific and targeted delivery of various therapeutic molecules, particularly crucial in the context of chemotherapeutic agents [28, 29]. Among the diverse nanoparticle (NP) delivery systems, liposomes have been pivotal in the field of anticancer drug delivery, representing the earliest investigated nanocarrier for this purpose. Niosomes, bilayer vesicles composed of non-ionic surfactants rather than phospholipids, offer advantages over liposomes in terms of chemical stability and production costs [24, 30].

In this study, two types of niosome formulations were prepared using microfluidic mixing with a pH gradient to facilitate the loading and delivery of DOX to cancer cells. The F1 formulation consisted of TW85 and CHOL in a 60:40 molar ratio, while the F2 formulation included TW85, CHOL, and DDAB in a 40:40:20 molar ratio.

Microfluidic mixing has recently gained widespread adoption for NP preparation due to its ability to rapidly develop particles with precise size and low size distribution, suitable for scalable production of NPs [31, 32].

Niosomes were initially prepared using ammonium sulfate (pH 4.5), followed by replacement of the external buffer with PBS (pH 7.4) after vesicle formation. This created niosomes with a pH gradient, where the internal aqueous compartment of the vesicles maintained pH 4.5 while the external environment was neutral. DOX was then incubated with these particles at approximately 60°C. Initially, DOX is un-ionized when exposed to the external neutral pH, facilitating its diffusion through the niosome bilayer membrane. Upon encountering the acidic pH of the niosome's internal aqueous compartment, DOX becomes ionized, effectively trapping it inside the niosomes where it cannot diffuse back out. This technique, extensively reported in literature for DOX loading into liposomes, was applied here for DOX loading into niosomes [33, 34]. The pH gradient method enhances DOX encapsulation into niosomes and minimizes drug leakage over time [35].

Niosomes characterization

Figure 1 presents the size, PDI, and ZP of niosomes as blank and DOX-loaded, measured by dynamic light scattering (DLS).

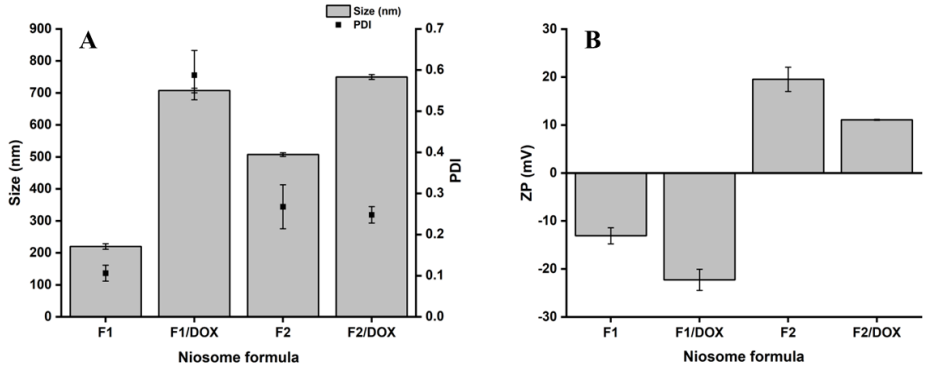

Figure 1. (A) size and PDI values and (B) ZP for both blank and DOX loaded niosomes. Results represent average ± standard deviation (SD) of three measurements.

As indicated in Figure 1, the size of the prepared formulations (F1 and F2) with or without DOX was uniform and monodisperse, as evidenced by the low PDI values for all formulations. Additionally, loading DOX into both formulations resulted in a significant increase in particle size due to the diffusion of DOX into the aqueous core of the niosomes.

Regarding surface charge, F1 exhibited a slightly negative charge, attributed to its composition of surfactant and cholesterol only, while F2 exhibited a cationic charge due to the presence of the cationic lipid DDAB. Loading DOX into F1 and F2 did not significantly alter the surface charges compared to blank particles, as DOX is encapsulated within the aqueous core of the particles and does not affect the surface charge. These size, PDI, and ZP results were monitored over three days, showing no significant changes (. These findings align with previous literature on niosome preparation using TW85 and DDAB via microfluidic mixing [31, 36-38].

Regarding particle morphology, TEM images in Figure 2 illustrate that blank particles of F1 and F2 were spherical in shape. Furthermore, TEM images clearly demonstrate that F1 niosomes were notably smaller than F2 niosomes.

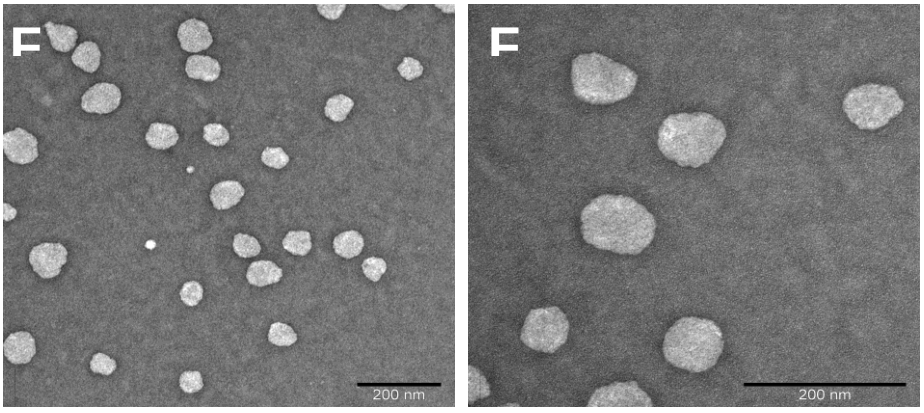

Figure 2. The TEM images presenting the morphology of blank F1 and F2 niosomes.

Doxorubicin Encapsulation Efficiency (EE%)

The EE% was calculated using the previously mentioned equation after constructing a standard calibration curve of absorbance versus concentration DOX, as shown in Figure 3. The percentages of DOX encapsulation efficiency in the niosome formulations are presented in Figure 4.

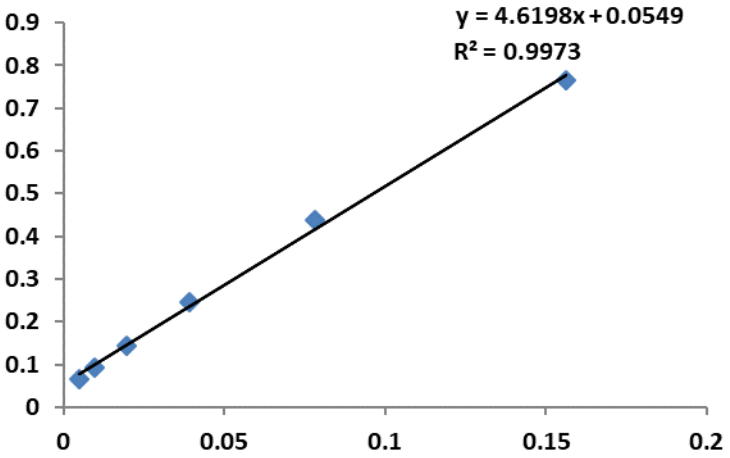

Figure 3. DOX standard curve measured by a plate reader at 480nm

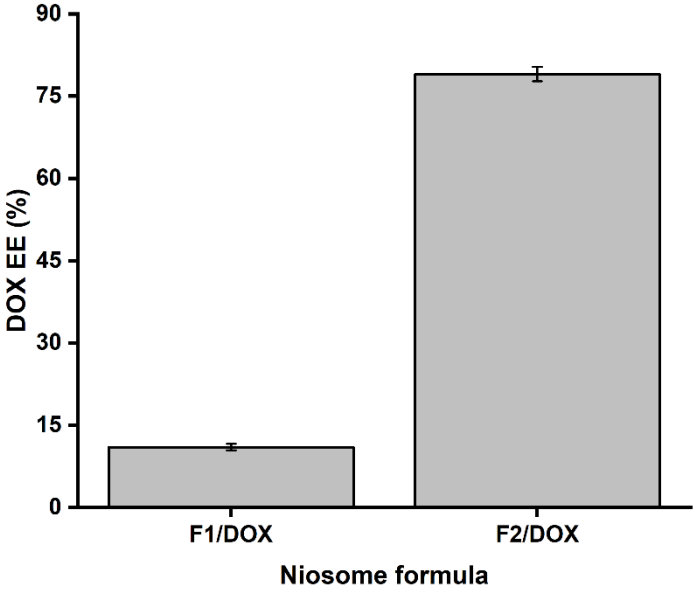

Figure 4. Percentage of encapsulation efficiency of DOX loaded in F1 and F2.

The efficacy of a NPs delivery system can be predicted based on several factors such as particle size, stability, and drug EE%. For niosomes prepared using microfluidic mixing and employing a pH gradient technique for DOX loading, the EE% determined using a DOX standard curve was approximately 11% for F1 and 79% for the cationic niosomes, F2.

Tevano et al. reported the preparation of niosomes composed of Pluronic L64 and Tween 60, resulting in DOX EE% of 55% and 64% for Tween 60 ~~niosomes~~ and L64 niosomes, respectively [39]. Here, it is evident that the inclusion of DDAB resulted in significantly higher DOX encapsulation compared to niosomes prepared using TW85 and cholesterol without DDAB. The primary distinction between the two formulations is the presence of DDAB, which had a pronounced effect on the final DOX encapsulation. The incorporation of DDAB likely facilitates the diffusion of the neutral form of DOX into the aqueous compartment of the niosomes, where it becomes protonated and trapped inside, preventing its leakage from within the particles [35].

Cytotoxicity effects of DOX loaded niosomes:

The use of niosomes for DOX delivery aims to reduce the side effects associated with DOX by enhancing its accumulation in cancer cells while minimizing distribution to normal cells. To assess the cytotoxicity of various DOX formulations, the biological activity of DOX-loaded niosomes was compared to free DOX against A549 and MCF7 cell lines using the MTT assay. Prior to DOX loading, the cytotoxicity of blank niosome particles was evaluated to determine non-toxic concentrations of each niosome formulation, ensuring any observed toxic effects on cells were attributable to DOX rather than the niosomes themselves. The IC_{50} values of free and loaded DOX were calculated using biograph software and are presented in Table 1.

Table 1. IC_{50} values of free and loaded DOX on A549 and MCF7 cells.

Treatment	IC_{50} on A549 (µg/ml)	IC_{50} on MCF7 (µg/ml)
Free DOX	0.369±0.090	0.214±0.080
F1/DOX	0.060±0.005	0.055±0.004
F2/DOX	1.622±0.096	3.282±0.104

The IC_{50} values of free DOX on both A549 and MCF7 cells were significantly reduced by loading DOX into F1 niosomes composed of TW85 and CHOL, indicating improved uptake of DOX by the cancer cells. However, loading DOX into F2 niosomes, composed of TW85, CHOL, and DDAB, resulted in a significant increase in the IC_{50} values of DOX on both cell lines. One possible explanation is the slow release of DOX from these cationic niosomes, implying that not all loaded DOX is readily available to induce cell death at the time of the MTT assay [40]. This slower response of cells to DOX loaded into F2 niosomes could potentially contribute to the long-term management of cancer. Despite the increased IC_{50} values observed for F2-loaded DOX, there remains a clear advantage in using niosomes for DOX delivery. They improve DOX targeting to cancer cells through passive targeting mechanisms and enhance permeability and retention effects, while mitigating the unwanted cardiac toxicity associated with free DOX. These observations warrant further investigation and experimentation to elucidate these results

The same IC_{50} values of free DOX have been consistently reported in the literature by several studies [41-43]. Furthermore, the enhancement of DOX efficacy by reducing its IC_{50} through loading into different types of nanocarriers has also been extensively documented. For instance, gold NPs have been utilized for DOX

loading and delivery, resulting in significantly greater activity against fibrosarcoma cancer. Gold NPs loaded with DOX achieved 81% tumor suppression, whereas free DOX achieved 48% tumor suppression in an in vivo model at a dose of 2.5 mg/kg, highlighting the potential for reducing DOX-associated cardiac toxicity [44, 45].

CONCLUSIONS

In conclusion, this study highlights the potential of niosomes as effective nanocarriers for the delivery of DOX in cancer therapy. Utilizing microfluidic mixing enabled the successful preparation of niosomes capable of encapsulating DOX and delivering it to A549 and MCF7 cancer cells. The findings demonstrate that niosomes can significantly reduce the concentration of DOX required for effective cancer cell suppression, thereby potentially mitigating its systemic toxicity. This approach not only enhances the therapeutic efficacy of DOX but also contributes to the broader development of nanocarrier-based formulations for delivering anticancer agents. Moving forward, further research and refinement of niosome formulations could lead to advanced strategies for improving cancer treatment outcomes while minimizing adverse effects.

Funding

No funding was received to assist with the preparation of this manuscript.

Statements and Declarations

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Ethical Approval

This declaration not applicable

REFERENCES

1. Senapati S., et al. Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduction and Targeted Therapy* 2018; 3(1):1–19.
2. Siegel R.L., Miller K.D. and Jemal A. Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians* 2019; 69(1):7–34.
3. Renu K., et al. Molecular mechanism of doxorubicin-induced cardiomyopathy—An update. *European Journal of Pharmacology* 2018; 818:241–253.
4. Pugazhendhi A., et al. Toxicity of doxorubicin (Dox) to different experimental organ systems. *Life Sciences* 2018; 200:26–30.
5. Bartlett J.J., Trivedi P.C. and Pulinilkunnil T. Autophagic dysregulation in doxorubicin cardiomyopathy. *Journal of Molecular and Cellular Cardiology* 2017; 104:1–8.
6. Johnson-Arbor K. and Dubey R. *Doxorubicin*. Treasure Island (FL): StatPearls Publishing; 2020.
7. Pawar S., Shevalkar G. and Vavia P. Glucosamine-anchored doxorubicin-loaded targeted nano-niosomes: Pharmacokinetic, toxicity and pharmacodynamic evaluation. *Journal of Drug Targeting* 2016; 24(8):730–743.
8. Jiang T., et al. Doxorubicin encapsulated in TPGS-modified 2D nanodisks overcomes multidrug resistance. *Chemistry – A European Journal* 2020; 26(11):2470–2477.
9. Ibraheem L.M. and Khattabi A.M. Studying the effect of functional group and size of silica nanoparticles loaded with quercetin on their in vitro characteristics. *Jordan Journal of Pharmaceutical Sciences* 2022; 15(4):569–582.
10. Wei Y., et al. Polydopamine and peptide-decorated doxorubicin-loaded mesoporous silica nanoparticles as a targeted drug delivery system for bladder cancer therapy. *Drug Delivery* 2017; 24(1):681–691.

11. Aljabali A.A., et al. Application of nanomaterials in the diagnosis and treatment of genetic disorders. In: *Applications of Nanomaterials in Human Health*. 2020:125–146.
12. Taymouri S. and Varshosaz J. Effect of different types of surfactants on the physical properties and stability of carvedilol nano-niosomes. *Advanced Biomedical Research* 2016; 5:1–9.
13. Matalqah S., Lafi Z. and Al-Kabariti A.Y. A recent review of PLGA-PEG hybrid nanoparticles for anticancer and anti-inflammatory applications. *Jordan Journal of Pharmaceutical Sciences* 2025; 18(1):180–195.
14. Obeid M.A., et al. Formulation and evaluation of nanosized hippadine-loaded niosome: Extraction and isolation, physicochemical properties, and in vitro cytotoxicity against human ovarian and skin cancer cell lines. *Journal of Drug Delivery Science and Technology* 2023; 104766.
15. Ge X., et al. Advances of non-ionic surfactant vesicles (niosomes) and their application in drug delivery. *Pharmaceutics* 2019; 11(2):55.
16. Takzare A., et al. *Trachyspermum copticum* essential oil incorporated niosome for cancer treatment. *Journal of Drug Delivery Science and Technology* 2019; 52:818–824.
17. Giordani S., et al. Liposomes characterization for market approval as pharmaceutical products: Analytical methods, guidelines and standardized protocols. *Journal of Pharmaceutical and Biomedical Analysis* 2023; 115751.
18. Kirpotin D.B., et al. Drug stability and minimized acid-/drug-catalyzed phospholipid degradation in liposomal irinotecan. *Journal of Pharmaceutical Sciences* 2023; 112(2):416–434.
19. Al-Kofahi T., et al. Paclitaxel-loaded niosomes in combination with metformin: Development, characterization and anticancer potentials. *Therapeutic Delivery* 2024; 15(2):109–118.
20. Obeid M.A., et al. Lipid-based nanoparticles for cancer treatment. In: *Lipid Nanocarriers for Drug Targeting*. Elsevier; 2018:313–359.
21. Kayani Z., Bordbar A.-K. and Firuzi O. Novel folic acid-conjugated doxorubicin-loaded β-lactoglobulin nanoparticles induce apoptosis in breast cancer cells. *Biomedicine & Pharmacotherapy* 2018; 107:945–956.
22. Verma S., et al. Metastatic breast cancer: The role of pegylated liposomal doxorubicin after conventional anthracyclines. *Cancer Treatment Reviews* 2008; 34(5):391–406.
23. Obeid M.A., et al. Use of nanoparticles in delivery of nucleic acids for melanoma treatment. In: *Melanoma: Methods and Protocols*. 2021:591–620.
24. Obeid M.A., Haifawi S. and Khadra I. The impact of solvent selection on the characteristics of niosome nanoparticles prepared by microfluidic mixing. *International Journal of Pharmaceutics: X* 2023; 5:100168.
25. Yaroslavov A., et al. A novel approach to a controlled opening of liposomes. *Colloids and Surfaces B: Biointerfaces* 2020; 190:110906.
26. Bindu C.H., et al. Nano-analytical techniques in pharmaceutical analysis. *Jordan Journal of Pharmaceutical Sciences* 2025; 18(2):341–376.
27. Kumar P., Nagarajan A. and Uchil P.D. Analysis of cell viability by the MTT assay. *Cold Spring Harbor Protocols* 2018; 2018(6):pdb.prot095505.
28. Bazak R., et al. Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. *Molecular and Clinical Oncology* 2014; 2(6):904–908.
29. Obeid M.A., et al. siRNA delivery to melanoma cells with cationic niosomes. In: *Melanoma: Methods and Protocols*. 2021:621–634.
30. Nasr M., et al. Vesicular aceclofenac systems: A comparative study between liposomes and niosomes. *Journal of Microencapsulation* 2008; 25(7):499–512.

31. Obeid M.A., et al. Microfluidic manufacturing of different niosome nanoparticles for curcumin encapsulation: Physical characteristics, encapsulation efficacy and drug release. *Beilstein Journal of Nanotechnology* 2019; 10:1826–1832.
32. Joshi S., et al. Comprehensive screening of drug encapsulation and co-encapsulation into niosomes produced using a microfluidic device. *Processes* 2020; 8(5):535.
33. Alyane M., Barratt G. and Lahouel M. Remote loading of doxorubicin into liposomes by transmembrane pH gradient to reduce toxicity toward H9c2 cells. *Saudi Pharmaceutical Journal* 2016; 24(2):165–175.
34. Al Qaraghuli M.M., et al. Control of malaria by bio-therapeutics and drug delivery systems. *Journal of Medical Microbiology & Diagnosis* 2017; 6(3).
35. Rehman A.U., et al. Development of doxorubicin hydrochloride-loaded pH-sensitive liposomes: Investigation of the impact of chemical nature of lipids and liposome composition on pH-sensitivity. *European Journal of Pharmaceutics and Biopharmaceutics* 2018; 133:331–338.
36. Barani M., et al. A new formulation of hydrophobin-coated niosome as a drug carrier to cancer cells. *Materials Science and Engineering: C* 2020; 110975.
37. Aljabali A.A., et al. A potential MRI agent and an anticancer drug encapsulated within CPMV virus-like particles. *Combinatorial Chemistry & High Throughput Screening* 2021; 24(10):1557–1571.
38. Gebril A., et al. Mucosal and systemic immune responses following mucosal immunisation of tetanus toxoid entrapped in lipid nanoparticles prepared by microwave reactor. *European Journal of Pharmaceutics and Biopharmaceutics* 2022; 171:11–18.
39. Tavano L., et al. Doxorubicin-loaded magneto-niosomes for targeted drug delivery. *Colloids and Surfaces B: Biointerfaces* 2013; 102:803–807.
40. Ibrahim M., et al. Encapsulation, release and cytotoxicity of doxorubicin loaded in liposomes, micelles and metal–organic frameworks: A review. *Pharmaceutics* 2022; 14(2):254.
41. Nunes A.S., et al. Establishment of 2D cell cultures derived from 3D MCF-7 spheroids displaying a doxorubicin-resistant profile. *Biotechnology Journal* 2019; 14(4):1800268.
42. Punia R., et al. Acacetin enhances the therapeutic efficacy of doxorubicin in non-small-cell lung carcinoma cells. *PLoS ONE* 2017; 12(8):e0182870.
43. Aljabali A.A., et al. Protein-based nanomaterials: A new tool for targeted drug delivery. *Therapeutic Delivery* 2022; 13(6):321–338.
44. Dhamecha D., et al. Doxorubicin-loaded gold nanoparticles: Implication of passive targeting on anticancer efficacy. *Pharmacological Research* 2016; 113:547–556.
45. Aljabali A.A., et al. Current state of, prospects for, and obstacles to mRNA vaccine development. *Drug Discovery Today* 2023; 28(2):103458.

تحضير، توصيف، وتقييم السمية الخلوية لجسيمات النيوزوم المحملة بدواء الدوكسوروبيسين والمحضّرة باستخدام تقنية المزج الميكروفلوي

محمد عبيد[1]*، سُجود التبينات[2]، حنين عماوي[3]، علاء الجبالي[1]، بهاء الطراد[2]

[1]قسم الصيدلانيات والتكنولوجيا الصيدلانية، كلية الصيدلة، جامعة اليرموك، إربد، الأردن.

[2]قسم العلوم البيولوجية، كلية العلوم، جامعة اليرموك، إربد، الأردن.

[3]قسم الصيدلة السريرية وممارسة الصيدلة، كلية الصيدلة، جامعة اليرموك، إربد، الأردن.

ملخص

يُعد دوكسوروبيسين من الأدوية الشائعة المضادة للسرطان، ويُستخدم لعلاج أنواع متعددة من الأورام. من أبرز عيوب هذا الدواء تطور مقاومة الخلايا السرطانية له وانتشاره الواسع في الجسم مما يؤدي إلى سُمية كبيرة. تُعد النيوزومات نوعًا من الجسيمات النانوية، تتكون من مواد غير أيونية وكوليسترول على شكل طبقة ثنائية. هدفت الدراسة الحالية إلى تطوير وتحسين نيوزومات محمّلة بالدوكسوروبيسين باستخدام تقنية المزج الميكروفلويدي من خلال استراتيجية تبديل المحاليل؛ وذلك لتقليل التوزيع غير المرغوب والسُمية المرتبطة بالدواء. تم تحضير تركيبين من النيوزومات F1 و F2 باستخدام البوليسوربيت 85 (T85) كمادة خافضة للتوتر السطحي غير الأيونية. تكوّن F1 من:T85 كوليسترول DOX/(40:60)، بينما تكوّن F2 من:T85 كوليسترول DDAB (40:40:20)/DOX.أظهرت F1 شحنة سطحية سالبة تقريبًا −13 مللي فولت، بينما أظهرت F2 شحنة موجبة تقدر بحوالي +20 مللي فولت. تم تحميل DOX بنجاح في كل من التركيبين، حيث بلغت كفاءة التحميل حوالي 11% لـ F1 و79% لـ F2. أدى تحميل DOX في F1 إلى انخفاض كبير في قيمة IC50 في خلايا MCF7 (سرطان الثدي) و A549(سرطان الرئة). بينما أدى تحميل DOX في F2 إلى ارتفاع قيمة IC50 في كلتا الخليتين مقارنة بالدواء الحر.

الاستنتاج: أظهر تركيب F1المحسن للنيوزومات المحملة بالدوكسوروبيسين تحسنًا في النشاط المضاد للسرطان مقارنة بالدواء في حالته الحرة. تشير هذه النتائج إلى أن النيوزومات المحسنة يمكن أن تكون أدوات فعالة لتوصيل دواء DOX إلى خلايا سرطان الثدي والرئة.

الكلمات الدالة: نيوزومات، دوكسوروبيسين، المزج الميكروفلويدي، تكنولوجيا النانو، توصيل الدواء.

* ***المؤلف المراسل: محمد عبيد***

m.obeid@yu.edu.jo

تاريخ استلام البحث 2024/08/24 وتاريخ قبوله للنشر 2024/11/03.