Nanomed. J. 10(1): 77-84, Winter 2023

RESEARCH PAPER

Synthesis of a stimuli-sensitive PEGylated nanoniosomal doxorubicin for the treatment of acute myeloid leukemia: An *in vitro* study

Fatemeh Bahrami-Banan [1], Mohammad Hasan Sheikhha [1], Nasrin Ghasemi [1], Masoumeh Rahmani [2], Mahdie Hemati [2], Mohammadreza Safdari [3, 4]*, Fatemeh Haghiralsadat [2]*

[1]Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Science, Yazd, Iran
[2]Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Orthopedic Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Orthopedic Surgery, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

ABSTRACT

Objective(s): One of the effective strategies for targeted chemotherapy of cancer is the use of lipid nanocarriers. In this study, an optimal formulation of niosomal drug containing doxorubicin was developed to monitor the potency against cancer cells.

Materials and Methods: In this experimental study, niosomal vesicles were prepared using phosphatidylcholine (20%), span60 (52.5%), cholesterol (22.5%), and DSPE-PEG2000 (5%) by the thin-film method. Doxorubicin was loaded into the niosomes using an inactive loading method.

Results: The features and characteristics of the nanocarrier were evaluated using Zeta-Sizer, SEM, FTIR, drug release, cellular uptake, and the cytotoxicity of the nanodrug carrier system by the MTT method. Niosomal vesicles-containing doxorubicin showed a size of ~156.8 nm, drug encapsulation efficiency of ~94.18%, zeta potential of ~-3.52 mV, and polydispersity index (PDI) of ~0.265. The prepared niosomes indicated a drug-controlled release system and FTIR analysis showed no interaction between nanocarriers containing drug and doxorubicin. Moreover, morphological examination of nanocarriers using SEM microscopy revealed that they had spherical structures. Also, cellular studies showed that drug toxicity was higher in encapsulated form of the drug compared with non-encapsulated doxorubicin which was confirmed by the cellular uptake results.

Conclusion: The results confirmed the proper physicochemical characteristics of these nanocarriers that significantly increased the toxicity of the encapsulated drug against the KG-1 cell line. It seems niosomal nanocarriers can be considered suitable carriers for drug delivery to cancer cells.

Keywords: *Acute myeloid leukemia, Doxorubicin, KG1 cell line, Niosomes*

How to cite this article
Bahrami-Banan F, Sheikhha MH, Ghasemi N, Rahmani M, Hemati M, Safdari MR, Haghiralsadat F. Synthesis of a stimuli-sensitive PEGylated nanoniosomal doxorubicin for the treatment of acute myeloid leukemia: An in vitro study. Nanomed J. 2023; 10(1): 77-84. DOI: 10.22038/NMJ.2022.69777.1745

INTRODUCTION

Drug delivery is one of the best methods to overcome the drug side effects and to improve the drug efficiency (1-3). Nanotechnology have attracted much attention in drug delivery due to its exceptional characteristics and features (4-7). Cancer is caused by the uncontrolled cell division and considered the most important causation of the death in the world (8-11). Acute myeloid leukemia (AML) is a hematopoietic stem cell malignant tumor characterized by the rapid growth of abnormal cells and heterogeneous clonal disorder (12). Anthracycline drugs are applied to a broad spectrum of various types of cancers impeding the tumor cell proliferation through intercalating DNA and blocking

* *Corresponding authors: Emails: safdari57@yahoo.com; fhaghirosadat@gmail.com*
Note. This manuscript was submitted on October 20, 2022; approved on December 31, 2022

topoisomerase II enzyme (13). Doxorubicin (DOX), an anthracycline antibiotic, is the most commonly used agent that is able to treat several types of cancers such as bladder, breast, osteosarcoma, lymphoma, and bone marrow cancer (14-16). However, its therapeutic index is restricted by unpleasant side effects such as cardiotoxicity, myelosuppression, and vomiting (17). Constructing a well-designed and specialized delivery system minimizes the drug absorption by normal cells leading to a decrease in undesirable physicochemical properties of drugs and increase in the penetration and survival of the drug in cancer cells which, in turn, enhances the therapeutic efficacy (18, 19).

Nanoniosomes are essentially non-ionic surfactant-based vesicles that control the release of drug in a sustained manner and increase the efficacy of the system for extended periods (20). Owing to their remarkable benefits, nanoniosomes are applied as a nanoreservoir/nanocarrier for lipophilic, hydrophilic and amphiphilic drugs that have caused considerable attention in drug delivery applications (21). Niosomes possessed simple procedure of synthesis, superior chemical stability, cost efficiency for application in drug delivery systems (22, 23). These are capable of entrapping hydrophilic molecules within an aqueous phase or alternatively hydrophobic molecules within lipid bilayers and preventing drugs from a rapid degradation which is resulted in the loss of pharmacological efficacy (24). This carrier system has been shown to have some remarkable advantages in comparison to the other types of nanocarriers because of more stability, low cost of production, excellent biocompatibility, and simple preparation (25). Incorporation of SPC80 (soybean phospholipids with 75% phosphatidylcholine) into formulations of nanoniosomes improves biocompatibility and biodegradability (26-28). In order to increase the stability (29, 30), half-life in systemic circulation (31), permeability (32), and retention effect (33), as well as decreasing the immunogenicity (34, 35), nanoniosomes are coated with polyethylene glycol (PEG) (36).

The primary goal of the present study is to obtain an optimal formulation of nanoniosomes containing DOX to minimize the dose of DOX to reach the maximum toxicity and effectivity for the elimination of cancer cells. A thermo-resonsive PEGylated nanoniosomal doxorubicin for the treatment of acute myeloid leukemia was developed. The physicochemical characteristics and in vitro drug release of the carrier in 37° C and 44° C were established. Finally, in vitro biological studies was performed that the results showed an excellent anti-cancer deference between the carrier and control.

MATERIALS AND METHODS

DOX hydrochloride (DOX-HCl) was obtained from Ebewe Pharma (Austria). Span60 was purchased from DaeJung Chemicals & Metals (SouthKorea).SPC80 (soybean phospholipids with 75% phosphatidylcholine) and 1,2-Distearoyl-sn-glycerol-3-phosphoethanolamine-N- [poly (ethylene glycol)2000] (DSPE-PEG2000) were obtained from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol and phosphate-buffered saline (PBS, pH=7.0) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Dialysis bags (MWCO 12000-14000) were supplied by Jingkehongda Biotechnology Co., Ltd. (Beijing, China) and DAPI (4',6-diamidino-2-phenylindole) was obtained from Thermo Fisher Scientific (Massachusetts, USA). Chloroform, ethanol and other chemicals used in this study were analytical grade and were used as received without any further purification.

Cell lines and preparation of biological samples

Bone marrow acute myeloblastic leukemia (AML) cell line KG-1 was supplied from the Pasteur Institute of Iran (Tehran, Iran) and cultured in RPMI-1640 medium (Gibco, Grand Island, USA) with 15% FBS (fetal bovine serum) (Gibco, Grand Island, USA) with penicillin-streptomycin (Gibco,Grand Island, USA) under the standard condition (37 °C and 5% CO_2 in a humidified incubator).

Preparation of niosomal DOX (Nio-DOX)

The present study was designed as an experimental study. DOX-containing nanoniosomes were synthesized and screened in terms of particle size, zeta potential, entrapment efficiency, and sustained release. In order to achieve the optimal parameters following experiments were carried out:

- Evaluation of the effect of different

cholesterol:span60 ratios.

- The preparation of nanoniosomes by addition of 5% synthetic DSPE-mPEG (2000)
- Determination of the cellular uptake, cytotoxicity and pharmacological efficacy of the optimized formulation.

We used the thin film method to prepare the nano-niosomes. In brief, Span60, cholesterol, SPC80, and DSPE-mPEG dissolved in to the chloroform, and the solvent was evaporated at 45˚C in a rotary evaporator. To ensure complete solvent removal, the thin film of lipid was aerated for several minutes with nitrogen gas, then drained onto paraffin and placed at 4 °C for 24 hours. Once prepared, the lipid-formed film was hydrated with DOX (0.5mg/ml) in phosphate-buffered saline (PBS) for 45 min at 55 °C. To reduce the size of niosomes micro tip probe sonicator (E–Chrom Tech Co, Taiwan) was used and then filtered with 0.22μm pore size polycarbonate membranes. Afterwards, unentrapped DOX was separated from Nio-DOX by dialysis bags possessed a cut-off of 12 kDa.

Determination of size, polydispersity index, and morphological characters

The particle size distribution and polydispersity index (PDI) of the niosomal vesicles were determined at 25 ˚C by dynamic light scattering (DLS) using a ZetaPALS zeta potential and particle size analyzer (Brookhaven Instruments, Holtsville, NY, USA). Each parameter was measured three times, after which we calculated the average values and standard deviations. Nanoniosomes were screened using a scanning electron microscope (SEM) (model KYKY-EM3200-30 kV, KYKY Technology Development Ltd., Beijing, China) and field-scanning electron microscopy to investigate the shape and structure of the nano-niosomes produced by the drug carrier (37-39).

Entrapment efficiency of DOX in nano-niosomes

To investigate the amount of accumulated doxorubicin, the Nio-DOX vesicles were mixed with isopropanol to break nanoniosomes and get free drugs. The encapsulation efficiency was determined by measuring absorption at 480 nm wavelength by UV-VIS spectrophotometer (model T80+, PG Instruments, UK) and finally the concentration of doxorubicin was obtained from the calibration equation (40).

The entrapment efficiencies were calculated based on the following formula:

Entrapment efficiency (%) = Loaded drug in nanoniosomes (mg/ml)/Total drug (mg/ml)×100

In vitro thermo- sensitive DOX release assay

A certain volume of doxorubicin hydrochloride-containing niosomes was poured into cellulose dialysis bag and the release of drugs was monitored in 10 ml of PBS solution (at 37 °C and 44 °C temperature) in shaking water bath at 75 rpm for 48 hours. Then, sampling was taken at specific times and substituted with an equal volume of fresh PBS. Depending on the calibration curve of doxorubicin in PBS, the concentration of drug was measured at different times. Samples were analyzed using the UV-VIS spectrophotometer at 480 nm. According to the total drug concentration of the niosomes formulation, the percentage of release was calculated at each time interval(41, 42).

Fourier transform infrared (FTIR) spectral evaluation

To analyze the molecular interaction between drug and nanocarriers, FTIR (Model 8300, Shimadzu Corporation, Tokyo, Japan) was applied for blank niosomes and niosomal-DOX. Samples were lyophilized and prepared as dry powder and mixed separately with potassium bromide (KBr), and the pellets were formed by placing samplesin a hydraulic press.

Nano-niosomal DOX cellular uptake experiments

KG1 cells were seeded at a density of 1.5 × 10^5 cells per well in a 6-well plate. After24 hours, the cells were treated with free DOX Nio-DOX. After 3 hours of incubation, the cells were washed with cold PBS twice and then fixed with a 4% paraformaldehyde solution (Sigma, USA). Next, the cells were stained with DAPI (Thermo Fisher Scientific, USA) at a concentration of 1mg/ml for 20 min. Images were obtained by fluorescence microscopy (Olympus, Japan).

Cytotoxicity study

Cell viability was measured with MTT assay. Cells were cultured in 96-well cell culture plates at a cellular density of 104 cells/well and incubated with blank niosomes, free DOX and Nio-DOX (a series of different DOX

concentrations) at 37 °C for 72 hours. In the next step, the contents of wells were removed and incubated with 20 µL MTT (5mg/ml) for 3 hours. After that, the supernatant was removed and the resultant formazan crystals were dissolved in 180 µL of DMSO. The results were measured by a microplate reader (synergy HTX, BioTek, USA) at 570 nm (42).

RESULTS AND DISCUSSION

The effect of span60: cholesterol molar ratio and DSPE- mPEG (2000) in niosomes formulation

In order to determine the optimal formulation in terms of having a small diameter, proper zeta potential, and high entrapment efficiency, various niosomal doxorubicin formulations were prepared. According to the results provided in Table 1, niosomes contained span60 and cholesterol at a molar ratio of 56:24 (F3) showed a higher drug encapsulation (86.05%) and smaller size (181.1 nm) compared with F1 and F2 formula. As shown in Table 1, the presence of PEGylation in the niosomal formulations led to an increase in drug encapsulation (94.18%) and a decrease in the mean size diameter. Thus, the optimal formula contained 52.5% span60, 22.5% cholesterol, 20% SPC80, and 5% DSPE-PEG was chosen as a selected formulation for further analysis.

Physical characterization: the optimum formulation

The size and polydispersity index (PDI) of the optimum formulation was measured. As shown in Fig. 1A and B, the mean size of Nio-DOX was 156.8 nm, and PDI was 0.265 showing monodispersity and homogeneity of nano-niosomes. Also, the zeta potential of the optimum formulation of Nio-DOX was about -3.52 mV. The SEM analysis of drug-containing nanoniosomes depicted a uniform spherical and structural shape. It also demonstrated that the mean diameter of the nanoniosomes was about 156.8 nm, which was consistent with the results of DLS (Fig.1C). In order to investigate the morphology of niosomes, FESEM was applied. As shown in Fig. 1D, the vesicles had a proper size distribution and a spherical shape.

Drug release profile

The results of DOX release from optimal nanoniosomes at 48 hours were represented in Fig. 2. The amount of DOX released from the nanoniosomal doxorubicin formulations in PBS buffer at body temperature and 44 °C (tumor tissue) was calculated using a standard curve of doxorubicin for 1, 2, 3, 4, 6, 8, 24, and 48 hours. The chart shows that the maximum amount of drug released from the niosomes for 48 hours at 37 °C is 38.38% and at 44 °C, about 58.24% in the neutral buffer.

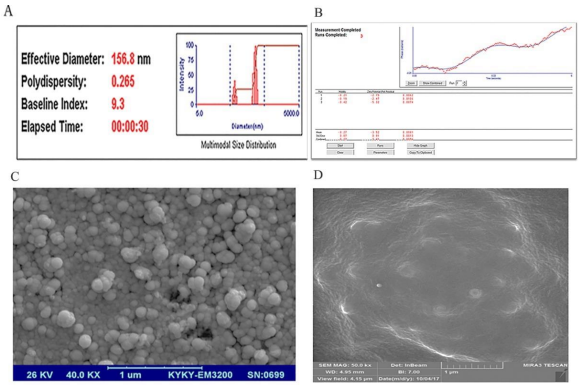

Fig. 1. A. Size distribution and B. zeta potential distribution of the optimum formula (F4). Morphological evaluation of niosomal doxorubicin (Nio-DOX) C. SEM and D. FESEM of optimum formula (F4)

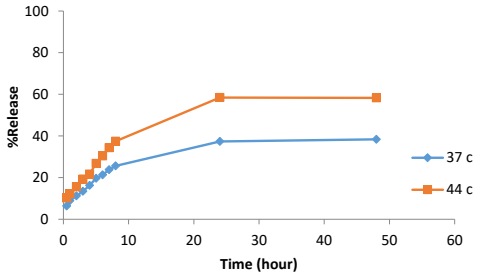

Fig. 2. *In vitro* kinetic release of drug in various temperature

Table 1. Effect of the non-ionic surfactant Span 60: cholesterol with various molar ratios and DSPE-mPEG (2000) on size, Zeta potential and entrapment efficiency (EE %), in doxorubicin loaded niosomes

Formula	Span-60 (%)	cholesterol (%)	SPC (%)	DSPE-mPEG (%)	Size (nm)	PDI	Zeta potential(mV)	Entrapment efficiency (EE%)
F1	72	8	20	0	264.8 ± 1.7	0.384 ± 0.03	-58.11 ± 1.02	78.73 ± 3.8
F2	64	16	20	0	243.1 ± 2.5	0.344 ± 0.01	-41.58 ± 0.84	81.99 ± 2.6
F3	56	24	20	0	181.1 ± 1.9	0.287 ± 0.04	-13.20 ± 0.77	86.05 ± 3.3
F4	52.5	22.5	20	5	156.8 ± 2.4	0 .265 ± 0.06	-3.52 ± 0.91	94.18 ± 4.1

FTIR spectral evaluation

The FTIR spectroscopy was established to study the interaction of DOX with the two-layer structure of the niosomes. The functional groups of the nanoniosomes surface produced by the FTIR spectroscopy were investigated. Fig. 3 shows the FTIR results of the optimal niosomal doxorubicin formula (F4). The peaks at 3241 cm-1 characterized the existence of the OH group, and 1632 cm-1 wave number was the tensile vibration characteristic of C=O in cholesterol, phospholipid, and span 60. As illustrated in Figure 3C, the out-of-plane bending peaks occurs within the range of 800–500 cm^{-1} that it can be used to assign mono-substitution on the DOX ring that affirms the entrapment of DOX with the niosome. As seen in Fig. 3, there is no new peak in FTIR spectrum of the niosome-containing drug system, and all peaks were repeated in FTIR spectrum of blank niosome and Nio-DOX indicated a lack of chemical interaction between the formulated system and DOX.

Nanoniosomal DOX cellular uptake experiments

Cellular uptake experiments were carried out to evaluate the cellular uptake behavior of free DOX and Nio-DOX. As illustrated in Fig. 4A, KG1 cell line treated with Nio-DOX showed a greater green and cyan (blue-green) color intensity compared to cells treated with free DOX. These results showed that the amount of DOX-loaded niosomes entered the cancer cells was higher than the free form of DOX. These findings have been corroborated with cytotoxicity assay.

Cytotoxicity study

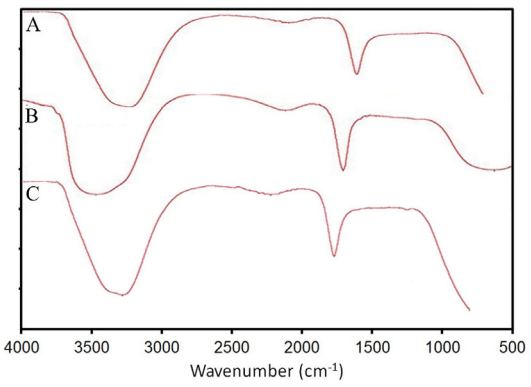

Fig. 3. FTIR spectra of optimum formula (F4): A) pure DOX, B) before drug loading, C) after drug loading

Fig. 4B shows the cell viability in the presence of blank niosome, free DOX, and Nio-DOX achieved by MTT assay for 48 hours. The results revealed that the blank niosome possessed a little cytotoxicity and the proliferation of KG-1 cell line was inhibited with free DOX and Nio-DOX. Of note, Nio-DOX had a higher cytotoxicity in comparison to the free form of DOX (P value <0.05). The results demonstrated DOX exerted the cytotoxicity in a dose-dependent manner in both forms of the free DOX and DOX-loaded niosomes.

AML is a cancer of the blood and bone marrow, with 13000 new cases per year, is prevalent in both of infants and older adults (43). In order to enhance the effectiveness of anti-leukemic drugs, high doses of anthracyclines should be used resulting in adverse side effects. Children are more prone to drug-induced cardiotoxicity than adults (44). To reduce the dose-limiting toxicity and optimize pharmacokinetics, drug delivery systems have been emerged to promote the biodistribution and chemical stability of anti-cancer agents. In this study, we have developed a novel nanoniosomal formulation to encapsulate DOX. These nanoniosomes

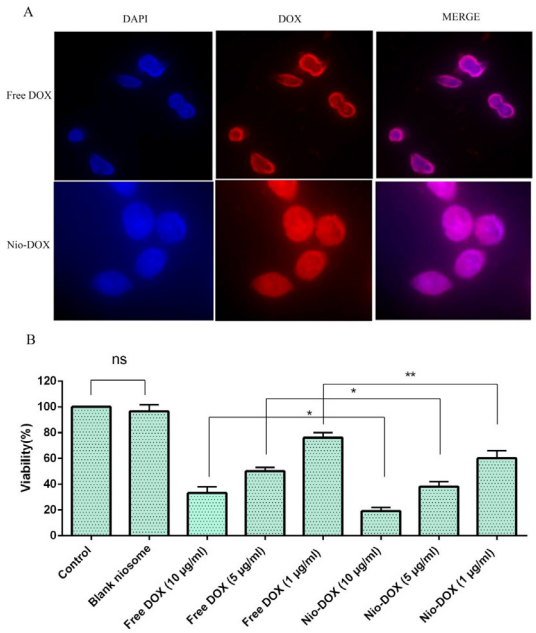

Fig. 4. A. Comparison of cellular uptake of Free DOX and Nio-DOX for the KG1 cells line. Cells were treated with DAPI for nucleus staining. Red: DOX fluorescence, Blue: fluorescence of the nucleus, Purple: Overlapping fluorescence. B. Cell viability on KG1 cell line of niosomal formulations with various concentrations of DOX, determined after 48 hours using MTT assay

were prepared from the nonionic surfactant Span-60. Span-60 exhibits a higher entrapment efficiency than Tween-60 possessing a high phase transition temperature and solidity at room temperature (45). The low hydrophile-lipophile balance (HLB) value of Span-60 plays a predominant role in the higher rate of DOX entrapment over that of Tween-60. In fact, a lower HLB value of the surfactant causes the higher entrapment efficiency and smaller vesicle size (46). The highest entrapment efficiency was found in formulation3 (F3) at a cholesterol/surfactant ratio of 24:56 (F3 *vs*. F1 and F2) (Table 1). An increase in cholesterol contents into theniosomal formulation enhances the encapsulation efficiency of DOX but declines the vesicular size (Table 1). Indeed, the addition of cholesterol increases the stability and the viscosity of the vesicle bilayer. So, the release and vesicle permeability are decreased (47). The presence of DSPE-mPEG 2000 in F4 improved the entrapment efficiency because it made nano-niosome more stable and decreased the drug permeability (48). Alternatively, the electrostatic repulsion among the niosomes with DSPE-PEG leads to a decrease in aggregation and the mean size (49). It has been postulated that the electrostatic repulsion among the niosomes with DSPE-PEG diminishes the possibility of the aggregation of niosomes after sonication, which causes the smaller average size. Regarding the small size of niosomes increases the blood levels of the niosomes the smaller size of thermo-sensitive niosomes incorporating DSPE-PEG may contribute to the elevation of systemic circulation time. It is known that nanoniosomes boost the cellular uptake of DOX indicating the preeminence of nanoniosomes endocytosis in comparison to free drug diffusion (50), and denoting further toxicity of Nio-DOX compared with free DOX. Correspondingly, the incorporation of free DOX into the nanoniosome formulations causes higher cytotoxicity effects. The continuous release, prevention of early deactivation of the drug, higher rate of drug entry into the cell make Nio-DOX more potent against the cancer cells than the free form of DOX. These results are congruous with the cellular uptake experiments and confirm that nanoniosomal doxorubicin formulations have a higher therapeutic efficacy in KG1 cells.

CONCLUSION

We successfully developed a novel DOX-loaded PEGylated nanoniosomes possessed nanoscale size, proper zeta potential, high encapsulation efficiency, and sustained drug release. When doxorubicin is encapsulated in nanoniosomes the pharmacokinetic of DOX is improved and the cellular uptake and cytotoxic activity are increased when compared with the free form of the drug. Overall, this drug-encapsulated nanocarrier system would be a promising strategy for battling the cancer cells.

ACKNOWLEDGMENTS

This research was funded by International Campus, Shahid Sadoughi University of Medical Science, Yazd, Iran. All authors read and approved the final manuscript, and have no conflict of interests.

REFERENCES

1. Haghiralsadat F, Amoabediny G, Naderinezhad S, Zandieh-Doulabi B, Forouzanfar T, Helder MN. Codelivery of doxorubicin and JIP1 siRNA with novel EphA2-targeted PEGylated cationic nanoliposomes to overcome osteosarcoma multidrug resistance. Int J Nanomedicine. 2018;13:3853.
2. Haghiralsadat F, Amoabediny G, Naderinezhad S, Forouzanfar T, Helder MN, Zandieh-Doulabi B. Preparation of PEGylated cationic nanoliposome-siRNA complexes for cancer therapy. Artif Cells Nanomed.Biotechnol. 2018;46(sup1):684-692.
3. Khatibi SA, Misaghi A, Moosavy MH, Basti AA, Koohi MK, Khosravi P, et al. Encapsulation of Zataria multiflora bioss. Essential oil into nanoliposomes and *in vitro* antibacterial activity against escherichia coli O157: H7. J Food Process Preserv. 2017;41(3):e12955.
4. Askari E, Naghib SM, Seyfoori A, Maleki A, Rahmanian M. Ultrasonic-assisted synthesis and *in vitro* biological assessments of a novel herceptin-stabilized graphene using three dimensional cell spheroid. Ultrason Sonochem. 2019;58:104615.
5. Pashazadeh-Panahi P, Belali S, Sohrabi H, Oroojalian F, Hashemzaei M, Mokhtarzadeh A, et al. Metal-organic frameworks conjugated with biomolecules as efficient platforms for development of biosensors. TrAC, Trends Anal Chem. 2021;141:116285.
6. Rashidi A, Omidi M, Choolaei M, Nazarzadeh M, Yadegari A, Haghierosadat F, et al., editors. Electromechanical properties of vertically aligned carbon nanotube. Adv Mat Res; 2013: Trans Tech Publ.
7. Fazaeli D, Mehrara R, Oroojalian F. Comparative efficacy of titanium dioxide nanoparticles loaded carboxymethyl cellulose and hydrogen peroxide gel on tooth whitening: An in-vitro study. Nanomed J. 2022;9(2):147-155.
8. Rahmanian M, Dehghan MM, Eini L, Naghib SM, Gholami H, Mohajeri SF, et al. Multifunctional gelatin–tricalcium phosphate porous nanocomposite scaffolds for tissue engineering and local drug delivery: *in vitro* and *in vivo* studies. J Taiwan Inst Chem Eng. 2019;101:214-220.
9. Salahandish R, Ghaffarinejad A, Naghib SM, Majidzadeh-A K, Zargartalebi H, Sanati-Nezhad A. Nano-biosensor for highly

sensitive detection of HER2 positive breast cancer. Biosens Bioelectron. 2018;117:104-111.
10. Gooneh-Farahani S, Naimi-Jamal MR, Naghib SM. Stimuli-responsive graphene-incorporated multifunctional chitosan for drug delivery applications: a review. Expert Opin Drug Deliv. 2019;16(1):79-99.
11. Azizi M, Tavana M, Farsi M, Oroojalian F. Yield performance of Lingzhi or Reishi medicinal mushroom, Ganoderma lucidum (W. Curt.: Fr.) P. Karst.(higher Basidiomycetes), using different waste materials as substrates. Int J Med Mushrooms. 2012;14(5).
12. Li S, Mason C, Melnick A. Genetic and Epigenetic Heterogeneity in Acute Myeloid Leukemia. Curr Opin Genet Dev. 2016;36:100-106.
13. Qu X, Wan C, Becker H-C, Zhong D, Zewail AH. The anticancer drug–DNA complex: Femtosecond primary dynamics for anthracycline antibiotics function. Proc Natl Acad Sci USA. 2001;98(25):14212-14217.
14. Hijiya N, Ness KK, Ribeiro RC, Hudson MM. Acute Leukemia as a Secondary Malignancy in Children and Adolescents: Current Findings and Issues. Cancer. 2009;115(1):23-35.
15. Hashemi M, Yadegari A, Yazdanpanah G, Omidi M, Jabbehdari S, Haghiralsadat F, et al. Normalization of doxorubicin release from graphene oxide: New approach for optimization of effective parameters on drug loading. Biotechnol Appl Biochem. 2017;64(3):433-442.
16. Haghiralsadat F, Amoabediny G, Naderinezhad S, Nazmi K, De Boer JP, Zandieh-Doulabi B, et al. EphA2 targeted doxorubicin-nanoliposomes for osteosarcoma treatment. Pharm Res. 2017;34(12):2891-2900.
17. Gindy ME, Prud'homme RK. Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. Expert Opin Drug Deliv. 2009;6(8):865-878.
18. Fernandez-Fernandez A, Manchanda R, McGoron AJ. Theranostic applications of nanomaterials in cancer: drug delivery, image-guided therapy, and multifunctional platforms. Appl Biochem Biotechnol. 2011;165(7-8):1628-51.
19. Abdel-Rashid RS, Abd Allah FI, Hassan AA, Hashim FM. Design, optimization, and in-vivo hypoglycemic effect of nanosized Glibenclamide for inhalation delivery. J Liposome Res. 2020(just-accepted):1-35.
20. Singh AP, Biswas A, Shukla A, Maiti P. Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. Signal Transduct Target Ther. 2019;4(1):1-21.
21. Ag Seleci D, Seleci M, Walter J-G, Stahl F, Scheper T. Niosomes as nanoparticular drug carriers: fundamentals and recent applications. J nanomater. 2016;2016.
22. De A, Venkatesh N, Senthil M, Sanapalli BKR, Shanmugham R, Karri VVSR. Smart niosomes of temozolomide for enhancement of brain targeting. Nanomed J. 2018;5:1849543518805355.
23. Moghassemi S, Hadjizadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. JCR. 2014;185:22-36.
24. Kamboj S, Saini V, Bala S. Formulation and Characterization of Drug Loaded Nonionic Surfactant Vesicles (Niosomes) for Oral Bioavailability Enhancement. Sci World J. 2014;2014:8.
25. Nazari-Vanani R, Karimian K, Azarpira N, Heli H. Capecitabine-loaded nanoniosomes and evaluation of anticancer efficacy. Artif Cells Nanomed Biotechnol. 2019;47(1):420-426.
26. Akbarzadeh I, Saremi Poor A, Yaghmaei S, Norouzian D, Noorbazargan H, Saffar S, et al. Niosomal delivery of simvastatin to MDA-MB-231 cancer cells. Drug Dev Ind Pharm. 2020;46(9):1535-1549.
27. Mirzaei-Parsa MJ, Najafabadi MRH, Haeri A, Zahmatkeshan M, Ebrahimi SA, Pazoki-Toroudi H, et al. Preparation, characterization, and evaluation of the anticancer activity of Artemether-loaded Nano-Niosomes against breast cancer. Breast Cancer. 2020;27(2):243-251.
28. Hurtado Y, Franco CA, Riazi M, Cortés FB. Improving the stability of nitrogen foams using silica nanoparticles coated with polyethylene glycol. J Mol Liq. 2020;300:112256.
29. Xue Y, Dong B, Liu X, Wang F, Yang J, Liu D. Using selenium-conjugated polyethylene glycol to enhance the stability of gold nanoparticles in biologically relevant samples. Sci China Chem. 2019;62(2):280-286.
30. Khosravi A, Baharifar H, Darvishi MH, Zarchi AAK. Investigation of chitosan-g-PEG grafted nanoparticles as a half-life enhancer carrier for tissue plasminogen activator delivery. IET Nanobiotechnol. 2020.
31. Shen C, Bian L, Zhang P, An B, Cui Z, Wang H, et al. Microstructure evolution of bonded water layer and morphology of grafting membrane with different polyethylene glycol length and their influence on permeability and anti-fouling capacity. J Membr Sci. 2020;601:117949.
32. Kostenko MO, Pokrovskiy OI, Zakhodyaeva YA, Voshkin AA, Lunin VV. Unusual effect of flow rate on retention in analytical supercritical fluid chromatography exemplified by polyethylene glycol separation. J Chromatogr A. 2020;1610:460513.
33. Baharifar H, Khoobi M, Arbabi Bidgoli S, Amani A. Preparation of PEG-grafted chitosan/streptokinase nanoparticles to improve biological half-life and reduce immunogenicity of the enzyme. Int J Biol Macromol. 2020;143:181-189.
34. d'Avanzo N, Celia C, Barone A, Carafa M, Di Marzio L, Santos HA, et al. Immunogenicity of Polyethylene Glycol Based Nanomedicines: Mechanisms, Clinical Implications and Systematic Approach. Adv Ther. 2020;3(3):1900170.
35. Kim JY, Kim JK, Park JS, Byun Y, Kim CK. The use of PEGylated liposomes to prolong circulation lifetimes of tissue plasminogen activator. Biomaterials. 2009;30(29):5751-5756.
36. Pishavar E, Oroojalian F, Ramezani M, Hashemi M. Cholesterol-conjugated PEGylated PAMAM as an efficient nanocarrier for plasmid encoding interleukin-12 immunogene delivery toward colon cancer cells. Biotechnol Prog. 2020;36(3):e2952.
37. Gholami Z, Dadmehr M, Jelodar NB, Hosseini M, Parizi AP. One-pot biosynthesis of CdS quantum dots through *in vitro* regeneration of hairy roots of Rhaphanus sativus L. and their apoptosis effect on MCF-7 and AGS cancerous human cell lines. Mater Res Express. 2020;7(1):015056.
38. Karimi MA, Dadmehr M, Hosseini M, Korouzhdehi B, Oroojalian F. Sensitive detection of methylated DNA and methyltransferase activity based on the lighting up of FAM-labeled DNA quenched fluorescence by gold nanoparticles. RSC Adv. 2019;9(21):12063-12069.
39. Ebrahimpour M, Akhlaghi M, Hemati M, Ghazanfary S, Shahriary S, Ghalekohneh SJ, et al. *In vitro* evaluation and comparison of anticancer, antimicrobial, and antifungal properties of thyme niosomes containing essential oil. Nanomed J. 2022;9(4).
40. Akhlaghi M, Taebpour M, Sharafaldini M, Javani O, Haghiralsadat BF, Oroojalian F, et al. Fabrication, characterization and evaluation of anti-cancer and antibacterial properties of nanosystems containing Hedera Helix aqueous extracts. Nanomed J. 2022;9(1):43-56.

41. Sekeres MA. Treatment of older adults with acute myeloid leukemia: state of the art and current perspectives. Haematologica. 2008;93(12):1769-1772.
42. Orgel E, Zung L, Ji L, Finklestein J, Feusner J, Freyer DR. Early cardiac outcomes following contemporary treatment for childhood acute myeloid leukemia: a North American perspective. Pediatr Blood cancer. 2013;60(9):1528-1533.
43. Ruckmani K, Sankar V. Formulation and Optimization of Zidovudine Niosomes. AAPS PharmSciTech. 2010;11(3):1119-1127.
44. Guinedi AS, Mortada ND, Mansour S, Hathout RM. Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int J Pharm. 2005;306(1-2):71-82.
45. Che J, Okeke CI, Hu ZB, Xu J. DSPE-PEG: a distinctive component in drug delivery system. Curr Pharm Des. 2015;21(12):1598-1605.
46. Hong MS, Lim SJ, Oh YK, Kim CK. pH-sensitive, serum-stable and long-circulating liposomes as a new drug delivery system. J Pharm Pharmacol. 2002;54(1):51-58.
47. Hemati M, Haghiralsadat F, Yazdian F, Jafari F, Moradi A, Malekpour-Dehkordi Z. Development and characterization of a novel cationic PEGylated niosome-encapsulated forms of doxorubicin, quercetin and siRNA for the treatment of cancer by using combination therapy. Artif Cells Nanomed Biotechnol. 2018:1-17.