Original Article *Iran J Ped Hematol Oncol. 2018, Vol 8. No 3, 153-160*

A Comparison between the Anticancer Activities of Free Paclitaxel and Paclitaxel-Loaded Niosome Nanoparticles on Human Acute Lymphoblastic Leukemia Cell Line Nalm-6

Ashraf Alemi PhD [1,*], Majid Farrokhifar PhD[2], Hadi Zare-Zardini PhD[3], Mojtaba Haghi Karamallah PhD[4]

1. Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Pediatrics, Sabzevar University of Medical Sciences, Sabzevar, Iran
3. Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Science, Yazd, Iran
*Corresponding author: Ashraf Alemi PhD, Department of Clinical Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Email: alemi.ashraf@gmail.com.

Received: 28 December 2017 **Accepted:** 14 March 2018

Abstract

Background: Niosomes or Nonionic surfactant vesicles are nano vehicles utilized in drug delivery systems, especially in cancer therapy. In this study, these vesicles were applied as delivery system for anticancer drug, paclitaxel and then, its anticancer activities was compared with free paclitaxel on Human Acute Lymphoblastic Leukemia (ALL) cell line Nalm-6.

Materialas and Methods: In this exprimental study, paclitaxel loaded niosome was prepared by thin film hydration method. The characterization tests included dynamic light scattering (DLS) and UV-Vis spectrophotometry were employed to evaluate the quality of the nanocarriers. Cytotoxicity of niosomal paclitaxel nanoparticles and free paclitaxel on human acute lymphoblastic leukemia cell line Nalm-6 after 24 hours were studied by MTT assay to determine cell viability.

Results: Percent of encapsulation paclitaxel prepared with sorbitane monostearate, cholesterol, and DSPE-mPEG 2000 was 97.21 %. In addition, the polydispersity index, mean size diameter, and zeta potentials of niosomal paclitaxel nanoparticles were found to be 0.244 ± 0.011, 106.3 ± 1.5 nm, and -26.03 ± 1.34; respectively. Paclitaxel released from nanoniosome in 72 h was 19.81 %. The results demonstrated that a 2.5~fold reduction in paclitaxel concentration was measured when the paclitaxel administered in nanoniosome compared to free paclitaxel solution in human acute lymphoblastic leukemia cell line Nalm-6.

Conclusion: As a result, the nanoparticle-based formulation of paclitaxel has high potential as an adjuvant therapy for clinical usage in human acute lymphoblastic leukemia therapy.

Keywords: Acute Lymphoblastic Leukemia, Anticancer, Nanoparticles, Niosome, Paclitaxel

Introduction

Cancer is one of the main causes of mortality worldwide and demonstrates a serious health problem (1). By 2050, according to the World Health Organization (WHO), it is anticipated that 17.5 million cancer deaths and 27 million new cancer cases will happen annually (2). Acute lymphoblastic leukemia (ALL) is a cancer of the lymphoid line of blood cells characterized by the development of large numbers of immature lymphocytes (3). ALL affected about 876,000 people globally in 2015 and resulted in about 111,000 deaths. It occurs most commonly in children, particularly those between the ages of 2 and 5. In the United States it is the most common cause of cancer and death from cancer among children. Chemotherapy is the initial treatment of choice, and most ALL patients receive a combination of medications (4). Paclitaxel (PTX) is a diterpenoid natural plant product (western yew, Taxus brevifolia) with a unique antineoplastic mechanism of action. PTX has been utilized for many years to treat ovarian, breast, lung, bladder, prostate, esophageal, pancreatic cancer, metastatic melanoma, and leukemia. PTX is a cell cycle-specific drug that binds with

high-affinity to microtubules, stabilizing and enhancing tubulin polymerization and suppressing spindle microtubule dynamics. Therefore, paclitaxel effectively inhibits mitosis, motility, and intracellular transport within cancerous cells, leading to apoptotic cell death (5-10). The clinical advances in paclitaxel are limited due to its formulation. Because of its low solubility, paclitaxel is formulated in a mixture of Cremophor EL/absolute ethanol (1:1 v/v). Cremophor EL is known to cause severe hypersensitivity reactions, yelosuppression, and peripheral neuropathy. As a result, various formulation strategies have been investigated to reduce vehicle-related side effects and meanwhile improve the chemotherapeutic efficacy of PTX (11-13). Much interest has been remarkably concentrated on developing nanotechnology-based PTX formulation such as polymeric micelles, liposomes, solid lipid nanoparticles, PTX-polymer conjugates, and emulsions (14-18). Nanotechnology has revolutionized diagnosis and treatment of cancer (19). Nano-sized drug delivery system (DDS) or nanocarrier is designed to deliver therapeutic and/or diagnostic agents to their target sites (20). Over the last decades, drug delivery systems using vesicular carriers have attracted great interest because these carriers provide high encapsulation efficiency, control drug release, enhance drug solubility, carry both hydrophilic and hydrophobic drugs, reduce side effects, prolong circulation in blood, and are able to target a specific area (21, 22). By definition, vesicles made of natural or synthetic phospholipids are called liposomes while transferosomes are modified liposomal systems that, in addition to phospholipids, contain a single chain surfactant as an edge activator. Ethosomes contain ethanol as an edge activator instead of a single chain surfactant. Despite having some advantages over conventional dosage forms, they have found many problems in practical applications such as high cost, utilize of organic solvent for preparation, and limited shelf life due to the lipids rancidification (23). Therefore, continuous endeavor is concentrated on finding an alternative vesicular carrier that is capable to provide adequate stability, biocompatibility, and lower toxicity. This requirement is accomplished by niosomes, emerging as an noteworthy candidate for drug delivery applications (24). Niosomes or non-ionic surfactant vesicles are uni- or multilamellar spheroidal structures. Niosomes are preferred as an effective alternative to conventional liposomes. They offer several advantages over liposome such as greater stability, lower cost, biodegradable, biocompatible, non-immunogenic, low toxicity, and lesser care in storage for industrial production in pharmaceutical applications. The Niosomal systems are considered to enhance the bioavailability of poorly water-soluble drugs such as paclitaxel (22, 25-29). The present study attempted to effectively deliver paclitaxel to human acute lymphoblastic leukemia cell line Nalm6 using niosomes in order to improve the solubility and the therapeutic effects of paclitaxel.

Materials and methods

2.1 Preparation of paclitaxel niosome by the thin film hydration method

The thin film hydration method employed to prepare niosome (30). Surfactant of sorbitane monostearate (Span 60) (Sigma Chemical Co St Louis, MO, USA), cholesterol (Sigma, USA) and polyethylene glycol (Lipoid PE 18 : 0/18 : 0– PEG2000, DSPE-mPEG 2000, Lipoid GmbH, Germany) (77:6:19.4:3 molar ratios) were dissolved in chloroform. Then, 0.5 $mg.mL^{-1}$ paclitaxel (Stragen, Switzerland) was added and the mixture was warmed to 45 ºC for 45 min. The solvent phase was evaporated on rotary evaporator until a thin-layered film formed. The thin film was hydrated with 3000µl distilled water at 55 ºC for 30 min.

The resultant suspensions were sonicated (model UP200St, Hielscher Ultrasonics GmbH, Germany) at 50 HTz and 4 ºC for 25 min in order to decrease niosomal particle size. Afterwards, free PTX (unloaded) was separated from niosomal PTX (Nio-PTX) by dialysis membrane (Jingkehongda Biotechnology Co., Ltd Beijing, China) that had a cut-off of 12 kDa.

2.2 Nanoparticle characterization

The Niosomal hydrodynamic diameters (particle size distribution), surface charges (zeta potential), and polydispersity index (PDI) were determined using dynamic light scattering technique (Zeta- Sizer instrument, DLS, Malvern Zatasizer Nano- ZS, Worcestershire, UK). Scattered light was detected at room temperature at an angle of 90°, and the diluted samples in 1700 µL of deionized water (0.1 mg mL^{-1}) were prepared and immediately measured after preparation. All measurements were carried out three times, and their mean values were calculated.

2.3 Determination of paclitaxel -loaded efficiency

In order to evaluate the entrapment efficiency, spectroscopic measurements were performed. The Nio-PTX particles were lysed with isopropanol to analyze PTX concentration. Nanoparticles were mixed with isopropanol to lyse the membranes and rapid shed of entrapped drugs. The amounts of PTX in niosome formulation were analyzed with a UV spectrophotometer (model T80+, PG Instruments, United Kingdom) at 236 nm (ʎmax) (24). A standard curve of PTX (0.2-2 mg.ml) in isopropanol was plotted at 236 nm to determine the correlation between the concentration of PTX and its absorbance with a dilution series of isopropanol solution of paclitaxel. All measurements were carried out three times, and their mean values were calculated. In order to evaluate the encapsulation efficiency, the following formula was used:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{The amount PTX in niosome (mg)}}{\text{Total drug of PTX added (mg)}} \times 100$$

2.4 In vitro release study

The release of paclitaxel from niosome was monitored by dialysis bag (MW cutoff = 12000 Da, Sigma, Germany) against PBS (dialysis medium) for three days at 37°C and pH 7.4. Then, 2 mL of samples were taken at different times (1,2,3,4,5,6,7,8,10,12,24,36,48, and 72 hours) and immediately replaced with the same volume of PBS to evaluate the PTX release rate from the niosome. Samples were analyzed using the UV spectrophotometer at 236 nm and the percentage of drug release was obtained using the PTX standard curve in PBS (0.2-2 mg.ml). All measurements were carried out three times, and their mean values were calculated.

2.5 Cell lines and culture conditions

Human acute lymphoblastic leukemia cell line Nalm-6 **(**was supplied from National Cell Bank, Pasteur Institute of Iran, Tehran, Iran) was cultured in RPMI-1640 medium (Gibco Invitrogen ,GmbH, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and penicillin G (100 U/ml)/streptomycin (100 µg/ml) at 37°C and 5% CO_2 maintained in an incubator under standard condition.

2.6 Cell viability studies

The cytotoxicity of niosomal formulation was determined by the MTT ((3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma, USA) assay (31, 32). Briefly, 1×10^4 Nalm-6 cells were plated in a total volume of 100 µl and suspended in medium with different concentration of free-PTX solution (0.4 - 0.003 mg.ml) and niosomal PTX(0.2 - 0.0007 mg.ml). After incubation for 24 h,

20 µl MTT (5 $mg.ml^{-1}$ in PBS) was added into each 96- well plate and incubated for three hour at 37 ˚C. Finally, 100 µl of DMSO was added to the each well to dissolve the formazan crystals. Absorbance of each well was recorded by EPOCH Microplate Spectrophotometer (synergy HTX, BioTek, USA) at 570 nm. The cytotoxicity of free-PTX solution and N- PTX was expressed as the inhibitory concentration (IC_{50}) value defined as the drug concentration required inhibiting cell growth by 50% relative to the control. The IC_{50} values of free PTX and N-PTX were calculated using GraphPad Prism software.

2.7. Statistical analysis

Statistical data analyses were performed via GraphPad Prism software and expressed as mean ± SD. The student t-test was used when comparing two independent groups. P-value < 0.05 was considered significant.

Results

3.1 Characterization of niosome formulation

The polydispersity index, mean size diameter, and zeta potential of niosomal paclitaxel nanoparticles prepared with span 60 , cholesterol and DSPE-mPEG 2000 were found to be 0.244 ± 0.011, 106.3 ± 1.5 nm, and -26.03 ± 1.34; respectively.

3.2 Encapsulation efficiency:

Encapsulation efficiency of the formulation was determined through paclitaxel standard curve in isopropanol (Figure 1). Percentage of encapsulation rate of paclitaxel in niosome nanoparticles was determined to be 97.21 ± 1.5 %.

3.3 In vitro release study

Using the standard curve of paclitaxel in PBS (Figure 2), the amount of released paclitaxel from PEGylated nanoniosomal formulation in PBS was calculated. After 72 h, 19.81 % of loaded PTX was released form PEGylated nanoniosomal formulation. The drug release profile increased with a gentle slope (Figure 3).

3.4 Evaluation of cellular cytotoxicity effect

It was observed that toxicity of paclitaxel in niosome formulation prepared with span 60, cholesterol, and DSPE-mPEG 2000 was higher than toxicity of free paclitaxel (Figure 4b and 4c, respectively). Furthermore, IC_{50} for niosome formulation was 8.52 ± 0.62 µg/ml, while the IC_{50} for free drug was determined to be 21.13 ± 0.5 µg/ml (Figure 5 and Figure 6, respectively). A 2.5~fold reduction in PTX concentration was measured when PTX was administered in nanoniosome compared to free PTX solution in Nalm-6 cells. The results showed that the paclitaxel niosome formula displayed significantly greater cytotoxic activity with human acute lymphoblastic leukemia cell line Nalm-6 in comparison with free paclitaxel.

Percentage of encapsulation was determined through paclitaxel standard curve in isopropanol (Figure 1). Percentage of encapsulation rate of paclitaxel in niosome nanoparticles was determined to be 97.21 ± 1.5 %.

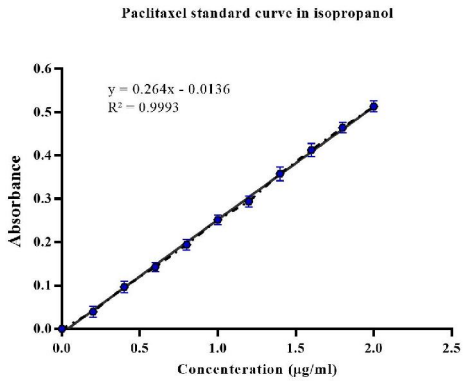


Figure 1. Paclitaxel standard curve in isopropanol (0.2-2 mg.ml) at 236 nm

All measurements were carried out three times, and their mean values were calculated.

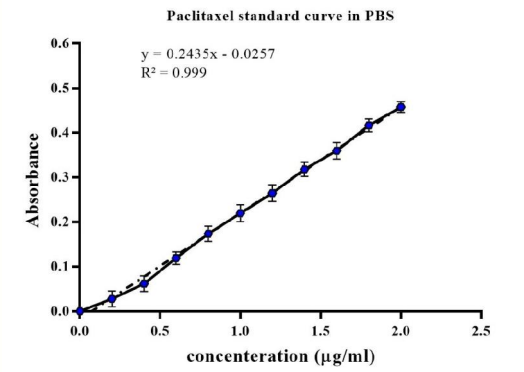


Figure2. Paclitaxel standard curve in PBS (0.2-2 mg.ml) at 236 nm

All measurements were carried out three times, and their mean values were calculated.

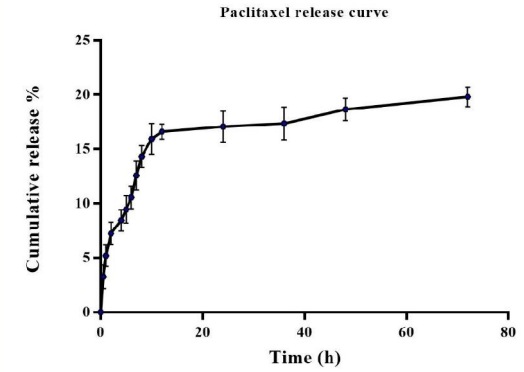
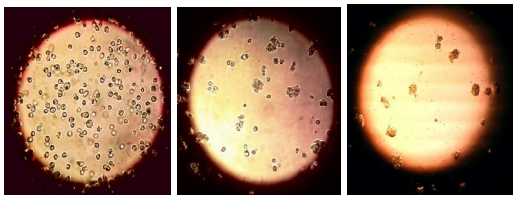


Figure 3. The in vitro release profile of paclitaxel from niosomal formulation at different times (1,2,3,4,5,6,7,8,10,12,24,36,48, and 72 hours) after drug encapsulation. All measurements were carried out three times, and their mean values were calculated.



a b c

Figure 4. Nalm-6 cell line: (a) before adding drugs, (b) after adding free PTX, (c) after adding Nio PTX

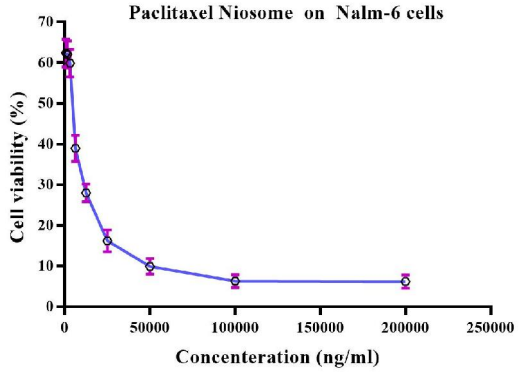


Figure 5. Cell viability assay of human acute lymphoblastic leukemia cell line Nalm-6 after 24 hours of treatment with various concentrations (0.2 - 0.0007 mg.ml) of paclitaxel niosome

All measurements were carried out three times, and their mean values were calculated.

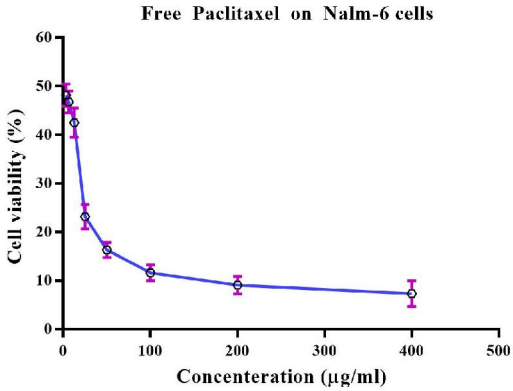


Figure 6. Cell viability assay of human acute lymphoblastic leukemia cell line Nalm-6 after 24 hours of treatment with various concentrations (0.4 - 0.003 mg.ml) of free paclitaxel

Discussion

It is specified that most of compounds prepared by combinatorial chemistry are compounds with low solubility which is considered a challenge in formulating these compounds. Using drug delivery carriers is an approach for improving solubility and maintaining physiological activity of such drugs. In this investigation, anticancer agent of paclitaxel was chosen as a model due to its low solubility in order to encapsulate in niosome nano carrier (22, 33). Nonionic

surfactants are the most common form of surfactant applied in preparing vesicles due to the superior advantages they endow with respect to stability, compatibility, and toxicity compared to their anionic, amphoteric or cationic counterparts. Nonionic surfactants are less toxic, less hemolytic, and less irritating to cellular surfaces. The hydrophilic–lipophilic balance (HLB) value of a surfactant lays a key role in controlling drug entrapment of the vesicle it forms. For a surfactant such as span 60 with an HLB value <6, cholesterol enhances stability of vesicles. It is also shown that cholesterol enables more hydrophobic surfactants to form vesicles, suppressing the tendency of the surfactant to form aggregates. The phase transition temperature (Tc) of the surfactant affects the entrapment efficiency. Thus, span 60 with a high Tc displays the highest entrapment efficiency (34, 35). In order to improve stability and circulation half-life, niosomes may be coated with appropriate polymer coatings such as polyethylene glycol (PEG), creating PEGylated niosomes (36). PEG coating also assists to reduce systemic phagocytosis, which results in lengthened systemic circulation, as well as reduced toxicity profiles (37, 38). The thin film hydration method used in this study to prepare niosomes was easy and had appropriate encapsulation efficiency and low cost benefit (39). PEG addition to niosomal formulation makes insoluble drugs, such as paclitaxel, soluble which results in encapsulation increment. This study also showed paclitaxel could highly be loaded into niosomes. It caused increase in bioavailability of drug. Moreover, nanoniosome was efficient in delivering the PTX drug to Nalm-6 cells. A 2.5~fold reduction in PTX concentration was measured when the PTX was administered in nanoniosome compared to free PTX solution in Nalm-6 cells. Finally, the obtained results suggested that niosomal nanoparticles can be used as an appropriate nanocarrier of paclitaxel for human acute lymphoblastic leukemia therapy and such formulation can be further in vivo studied.

Conclusions

Our results attained successful formulation of paclitaxel niosome that contained span 60, cholesterol and DSPE-mPEG 2000 by thin film hydration method, which could encapsulate PTX (0.5 mg/ml) with 97.21 % efficiency. The resulting paclitaxel niosome had a suitable particle size of 106.3 nm, adequately controlled PTX release (19.81% PTX after 72 hours) and increased tumor cell kill. Hence, the nanoparticle-based formulation of paclitaxel has high potential as an adjuvant therapy for clinical usage in human acute lymphoblastic leukemia therapy.

Conflict of interest

The author(s) declare that they have no conflict of interests

References

1.Vinardell M, Mitjans M. Antitumor Activities of Metal Oxide Nanoparticles. Nanomaterials. 2015;5(2):1004.

2.Saraswathy M, Gong S. Different strategies to overcome multidrug resistance in cancer. Biotechnology Advances. 2013;31(8):1397-407.

3.Hunger SP, Mullighan CG. Acute Lymphoblastic Leukemia in Children. The New England journal of medicine. 2015;373(16):1541-52.

4.Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381(9881):10.1016/S0140-6736(12)62187-4.

5.Boztas AO, Karakuzu O, Galante G, Ugur Z, Kocabas F, Altuntas CZ, et al. Synergistic interaction of paclitaxel and curcumin with cyclodextrin polymer complexation in human cancer cells. Molecular pharmaceutics. 2013;10(7):2676-83.

6.Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nature reviews Cancer. 2004;4(4):253-65.

7.Lieu CH, Chang YN, Lai YK. Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. Biochem Pharmacol. 1997;53(11):1587-96.
8.Lu KH, Lin KL, Yang CC, Hsia TC, Hsiao YM, Chou MC, et al. The effect of paclitaxel on gene expression and activity of arylamine N-acetyltransferase and DNA-2-aminofluorene adduct formation in human leukemia HL-60 cells. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2002;40(5):705-13.
9.Ma P, Mumper RJ. Paclitaxel Nano-Delivery Systems: A Comprehensive Review. Journal of nanomedicine & nanotechnology. 2013;4(2):1000164.
10.Rowinsky EK, Burke PJ, Karp JE, Tucker RW, Ettinger DS, Donehower RC. Phase I and pharmacodynamic study of taxol in refractory acute leukemias. Cancer research. 1989;49(16):4640-7.
11.Gupta N, Hatoum H, Dy GK. First line treatment of advanced non-small-cell lung cancer - specific focus on albumin bound paclitaxel. Int J Nanomedicine. 2014;9:209-21.
12.Ma P, Mumper RJ. Paclitaxel Nano-Delivery Systems: A Comprehensive Review. Journal of nanomedicine & nanotechnology. 2013;4(2):1000164.
13.Zou H, Li L, Garcia Carcedo I, Xu ZP, Monteiro M, Gu W. Synergistic inhibition of colon cancer cell growth with nanoemulsion-loaded paclitaxel and PI3K/mTOR dual inhibitor BEZ235 through apoptosis. International Journal of Nanomedicine. 2016;11:1947-58.
14.Chen D-B, Yang T-z, Lu W-L, Zhang Q. In Vitro and in Vivo Study of Two Types of Long-Circulating Solid Lipid Nanoparticles Containing Paclitaxel. Chemical and Pharmaceutical Bulletin. 2001;49(11):1444-7.
15.Ruttala HB, Ko YT. Liposome Encapsulated Albumin-Paclitaxel Nanoparticle for Enhanced Antitumor Efficacy. Pharmaceutical Research. 2015;32(3):1002-16.
16.Xie Z, Guan H, Chen X, Lu C, Chen L, Hu X, et al. A novel polymer-paclitaxel conjugate based on amphiphilic triblock copolymer. Journal of controlled release : official journal of the Controlled Release Society. 2007;117(2):210-6.
17.Wang X, Zhou J, Wang Y, Zhu Z, Lu Y, Wei Y, et al. A phase I clinical and pharmacokinetic study of paclitaxel liposome infused in non-small cell lung cancer patients with malignant pleural effusions. European Journal of Cancer. 2010;46(8):1474-80.
18.Xiao K, Luo J, Fowler WL, Li Y, Lee JS, Xing L, et al. A self-assembling nanoparticle for paclitaxel delivery in ovarian cancer. Biomaterials. 2009;30(30):6006-16.
19.Jaishree V, Gupta PD. Nanotechnology: A Revolution in Cancer Diagnosis. Indian Journal of Clinical Biochemistry. 2012;27(3):214-20.
20.Eldar-Boock A, Polyak D, Scomparin A, Satchi-Fainaro R. Nano-sized polymers and liposomes designed to deliver combination therapy for cancer. Current opinion in biotechnology. 2013;24(4):682-9.
21.Jain S, Jain V, Mahajan SC. Lipid Based Vesicular Drug Delivery Systems. Advances in Pharmaceutics. 2014;2014:12.
22.Sezgin-Bayindir Z, Yuksel N. Investigation of Formulation Variables and Excipient Interaction on the Production of Niosomes. AAPS PharmSciTech. 2012;13(3):826-35.
23.Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta Pharmaceutica Sinica B. 2011;1(4):208-19.
24.Sharma V, Anandhakumar S, Sasidharan M. Self-degrading niosomes for encapsulation of hydrophilic and hydrophobic drugs: An efficient carrier for cancer multi-drug delivery. Materials

Science and Engineering: C. 2015;56:393-400.
25.Abdelbary AA, AbouGhaly MH. Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: application of Box-Behnken design, in-vitro evaluation and in-vivo skin deposition study. International journal of pharmaceutics. 2015;485(1-2):235-43.
26.Shilakari Asthana G, Sharma PK, Asthana A. In Vitro and In Vivo Evaluation of Niosomal Formulation for Controlled Delivery of Clarithromycin. Scientifica. 2016;2016:6492953.
27.Tavano L, Aiello R, Ioele G, Picci N, Muzzalupo R. Niosomes from glucuronic acid-based surfactant as new carriers for cancer therapy: preparation, characterization and biological properties. Colloids and surfaces B, Biointerfaces. 2014;118:7-13.
28.Tavano L, Muzzalupo R, Picci N, de Cindio B. Co-encapsulation of antioxidants into niosomal carriers: gastrointestinal release studies for nutraceutical applications. Colloids and surfaces B, Biointerfaces. 2014;114:82-8.
29.Tavano L, Muzzalupo R, Picci N, de Cindio B. Co-encapsulation of lipophilic antioxidants into niosomal carriers: percutaneous permeation studies for cosmeceutical applications. Colloids and surfaces B, Biointerfaces. 2014;114:144-9.
30.Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. International journal of pharmaceutics. 1998;172(1):33-70.
31.Baek JS, Cho CW. Controlled release and reversal of multidrug resistance by co-encapsulation of paclitaxel and verapamil in solid lipid nanoparticles. International journal of pharmaceutics. 2015;478(2):617-24.
32.Baek JS, Cho CW. A multifunctional lipid nanoparticle for co-delivery of paclitaxel and curcumin for targeted delivery and enhanced cytotoxicity in multidrug resistant breast cancer cells. Oncotarget. 2017;8(18):30369-82.
33.Sezgin-Bayindir Z, Onay-Besikci A, Vural N, Yuksel N. Niosomes encapsulating paclitaxel for oral bioavailability enhancement: preparation, characterization, pharmacokinetics and biodistribution. Journal of microencapsulation. 2013;30(8):796-804.
34.Khan R, Irchhaiya,R. Niosomes: a potential tool for novel drug delivery. Journal of Pharmaceutical Investigation 2016;46:195–204.
35.Kumar G, Rajeshwarrao, P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta Pharmaceutica Sinica B. 2011;1(4):208-19.
36.Elliott JA. PEGylation of Niosomes: University of South Florida; 2009.
37.Wang AZ, Langer R, Farokhzad OC. Nanoparticle delivery of cancer drugs. Annual review of medicine. 2012;63:185-98.
38.Ahmed M, Moussa M, Goldberg SN. Synergy in cancer treatment between liposomal chemotherapeutics and thermal ablation. Chemistry and physics of lipids. 2012;165(4):424-37.
39.Marianecci C, Di Marzio L, Rinaldi F, Celia C, Paolino D, Alhaique F, et al. Niosomes from 80s to present: The state of the art. Advances in Colloid and Interface Science. 2014;205:187-206.