IUFS Journal of Biology
IUFS J Biol 2014, 73(1): 9-19

Research Article

Poly (DL-Lactic-Co-Glycolic Acid) Microparticle-Doxorubicin Formulations for Anti-cancer Drug Delivery

Gülseren Petek Çağlar [1], Serap Yalçın [2], Güngör Gündüz [3], Ufuk Gündüz[1]*

[1]*Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey*
[2]*Department of Food Engineering, Ahi Evran Üniversity, Kırşehir 40100, Turkey*
[3]*Department of Chemical Engineering, Middle East Technical University, Ankara 06800, Turkey*

Abstract

Cancer is a group of diseases in which normal cells are converted to cells capable of autonomous growth and invasion. In the chemotherapeutic control of cancer, drugs are usually given systemically so they reach toxic levels in cancer cells. This causes serious side effects in healthy cells. Another important problem with chemotherapy is resistance developed to cytotoxic drugs (multi drug resistance). As a possible solution to these problems, in the present study, two different microparticle fabrication methods were compared; double emulsion solvent evaporation (SE) method for encapsulation of Doxorubicin into PLGA microparticles. The most appropriate method was the SE techniquies which lead to higher encapsulation efficiencies. Processing factors were evaluated for their effects on encapsulation efficiency and results indicated that any change that hinder drug diffusion would result in increased drug content in microparticles. To asses the cytotoxicities of synthesized microparticles, cell proliferation assays were performed with XTT reagent on Doxorubicin resitant and sensitive breast cancer cell lines, MCF-7. DOX entrapped microparticles was effective on both sensitive and DOX resistant MCF-7 cells. The concentration of drug in resistant cancer cells was increased indicating a partial reversal of drug resistance. The results of this study will provide new insights to the development of new drug delivery systems for cancer therapy.

Keywords: PLGA, DOX, MCF-7, microparticle, drug delivery.
*Corresponding Author: Ufuk Gündüz (e-mail:ufukg@metu.edu.tr)
(Received: 05.11.2013 Accepted: 21.04.2014)

Anti-kanser İlaç Taşınması için Doksorubisin-Poli (DL-Laktik-Ko-Glikolik Asit) Mikroparçacıklarının Formülasyonları

Özet

Kanser, hücrelerin kontrolsuz çoğalma ve yayılma özelliği kazandığı bir hastalıktır. Kanserin kemoterapi ile tedavisinde ilaçlar genelde sistemik olarak verilir ve kanser hücrelerinde toksik etkiye ulaşır. Ancak kanserli hücrelerin yanı sıra sağlıklı hücrelerde de ilaç seviyesi toksik düzeylere ulaştığında ciddi yan etkilere neden olmaktadır. Kemoterapi ile ilgili bir başka önemli problemde anti-kanser ilaçlara karşı gelişen dirençliliktir (çoklu ilaç dirençliliği). Bu problemleri çözmek için, çalışmada iki farklı mikroparçacık üretim metodu karşılaştırılmıştır; PLGA mikroparçacıklarının içine, Doksorubisin tekli emulsiyon çözücü buharlaştırma (SE) yöntemi ile tutuklanmıştır. En yüksek ilaç tutuklanma yüzdesi SE tekniği ile elde edilmiştir. Sentezlenen mikroparçacıklar sitotoksisitesini belirlemek için, hücre çoğalma testi, XTT kullanılarak Doksorubisin dirençli ve duyarlı meme kanseri hücre hatlarında, MCF-7, kullanılmıştır. Doksorubisin tutuklanmış mikroparçacıklar hem duyarlı hemde Doksorubisin dirençli MCF-7 hücrelerinde etkili olmuştur. Dirençli kanser hücrelerinde ilacın konsantrasyonu kısmi olarak ilaç dirençliliğinin geri çevrilmesini artırmıştır. Bu çalışmanın sonuçları, kanser tedavisi için yeni ilaç taşıma sistemlerinin geliştirilmesine katkı sağlayacaktır.

Anahtar Kelimeler: PLGA, Doksorubisin, MCF-7, mikroparçacık, ilaç taşınması

Introduction

The synthesis and characterization of micro- or nano- particles have been the focus of an intensive recent years. Research has been done on the application of various polymers in drug delivery systems. Micro- or nano- particles provide a better accumulation in tumor tissues through an enhanced permeability and retention (EPR) effect (Iyer et al. 2006), ability to overcome multidrug resistance (Jabr-Milane et al. 2008), and better pharmacokinetics of drug *in vivo* (Cao and Feng 2008). Polymer coated micro- or nano- particles have been extensively employed as effective drug delivery systems to enhance the efficacy and safety of encapsulated drugs. Among those polymers, poly (dl-lactide-co-glycolide) (PLGA) is commonly used in biomedical industry as a major component due to its biocompatibility and biodegradability (Bilati et al. 2005).

In this study, two different methods for preparation of PLGA MPs for Doxorubicin (DOX) delivery. Encapsulation and release of DOX was studied. The effects of various formulation parameters on characteristics and drug encapsulation of microparticles were investigated.

DOX, an anthracycline antibiotic induces cardiac toxicity which is associated with a high incidence of morbidity and mortality (Phillips et al. 2008; Takemura and Fujiwara 2007). Besides cardio toxicity, like many other anticancer drugs targeting proliferating cell populations, DOX causes extravasations and necrosis at the injection site, significant gastrointestinal toxicity, with nausea, vomiting, diarrhea, and stomatitis (Gabizon et al. 1994). Encapsulating DOX inside a polymer matrix which allows sustained relase of the drug will decrease the DOX induced cardiac toxicity by avoiding high peak plasma concentrations. In addition, a biocompatible polymer will shield the drug and limit the contact of the drug with healthy cells. Moreover, resistance of malignant tumors to DOX is another important cause of treatment failure in patients with cancer. The best-studied mechanism of MDR (Multı Drug Resistance) is the overexpression of an energy-dependent multidrug efflux pump, known as the multidrug transporter P-glycoprotein (P-gp) (Ambudkar et al. 1999). Encapsulation of DOX into microparticles may also overcome the resistance problem.

In the present study, DOX is encapsulated in PLGA micro particles by single (o/w) and double microemulsion (w/o/w) solvent evaporation techniques. In order to optimize the process, different formulations are prepared and investigated for their encapsulation efficiency, size distribution, and zeta potential.

Materials and methods

Materials

The biodegradable polymer poly (d,l-lactic-co-glycolic acid) (PLGA) used in encapsulation studies had a feed ratio of lactide to glycolide 75:25 with an average molecular weight (Mw) of 66,000-107,000, and was purchased from Sigma (Germany). Polyvinyl alcohol (PVA) (MW: 30,000-70,000), Phosphate buffered saline (PBS) tablets were obtained from Sigma-Aldrich (Germany). Dialysis membranes (MwCo 1000, Diameter 26mm) were obtained from Serva (Germany).

For cell culture, 0.25% Trypsin-EDTA solution, gentamycin sulphate, 0.5% tryphan blue solution, XTT cell proliferation kit were purchased from Biological Industries, Kibbutz Beit Haemek (Israel). RPMI 1640 medium (2.0g/l $NaHCO_3$ stable glutamine), fetal bovine serum (FBS) were obtained from Biochrom Ag. (Germany). Dimethylsulfoxide (cell culture grade), sodium dodecyl sulfate (molecular biology grade) were obtained from AppliChem (Germany). MCF-7 monolayer type human epithelial breast adenocarcinoma cell line was provided from Food and Mouth Diseases Institute (Şap) (Ankara). DOX. HCl (579.98g/mol) was kindly donated by Prof. Dr. Fikret Arpaci and Prof. Dr. Ali Uğur Ural, Gülhane Military Medical School Hospital (Ankara).

Microparticle formation

DOX was encapsulated in PLGA microparticles by single (SE) (O/W) and double (DE) (W/O/W) micro-emulsion techniques (Bilati et al. 2005). Several process factors were optimized to achieve maximum entrapment efficiency. The best formulations with the highest drug encapsulation efficiencies were further investigated with respect to their size distribution, zeta potential, and drug release profiles.

W/O/W double emulsion (DE) solvent evaporation method

Every step of the encapsulation process was performed under laminar flow hood using sterile materials. DOX was dissolved in sterile water. PLGA was dissolved in chloroform to obtain the organic phase. Aqueous DOX solution was added into the organic phase and sonicated. This resulted in the first W/O emulsion phase. PVA surfactant solution was filtered through 20 µm filter by using a syringe. Every material was pre-cooled to 4°C before encapsulation. In cold room (4°C), this primary emulsion was added drop wise by using a sterile syringe into the outer water phase under magnetic stirring at 1000 rpm. This emulsion was stirred for 10 minutes. Then the cap was opened and chloroform was evaporated completely. As the solvent evaporated, microparticles were obtained. Microparticles were harvested by centrifugation and washed extensively to remove any residual-free drugs and -free surfactants. The PLGA microparticles were then lyophilized overnight, and kept in a sealed container at -80°C until use.

Unless otherwise mentioned, all the experiments were conducted by varying one of the parameters while keeping other processing parameters at a set of standard conditions: 20 ml 2% PVA solution the aqueous phase, 25 mg/ml PLGA in chloroform as the organic phase. DOX was dissolved in sterile distilled water. Theoretical drug loading was calculated by (TL) dividing the drug amount to PLGA amount and drug amount and kept at 1%. Sonication of the first emulsion was done until the solution becomes homogenous. Speed and duration of centrifuge for microparticle collection was 1000 rpm for 15 minutes.

O/W single emulsion (SE) solvent evaporation method

DOX was solubilized in chloroform ($CHCl_3$) containing 5M equivalent of Triethylamine (TEA) in respect to DOX at 1 mg/ml concentration. Solution was sonicated. PLGA was dissolved in this solution to obtain the oil phase. This was added to the outer water phase as explained. All the other steps of microparticle preparation were same with double emulsion method. Standard conditions: 20 ml 2% PVA solution as the aqueous phase, 8 mg/ml PLGA and 1 mg/ml DOX were dissolved in chloroform as the organic phase. Theoretical drug loading was 20%, sonication was done until the solution becomes homogenous, speed and duration of centrifuge for microparticle collection was 1000 rpm for 15 minutes. Theoretical loading was 5% and emulsification steps were performed at 4^0C.

Optimization of encapsulation process parameters

In order to test the effect of concentration of the surfactant on encapsulation efficiency (EE), every experimental parameter except PVA concentration were kept constant. PVA concentration was changed from 1% to 2%. To test the effect of volume ratio of the organic phase to the inner water phase (Vo/Vw1) on EE, instead of 500 µl, same amount of drug was dissolved in 50 µl. In the first case Vo/Vw1 ratio was 5 (1000 µl chloroform / 500 µl water) and in the second case it was 20 (1000 µl chloroform / 50 µl water). To test the effect of solvent evaporation time on EE, instead of overnight solvent evaporation, solvent was only evaporated until no more phase difference was seen (approximately 3 hours). To test the effect of emulsification temperature on EE, DOX and PLGA solutions were mixed and allowed to be stirred under magnetic stirring for 5 hours at room temperature (RT).

Determination of drug encapsulation efficiency

The drug content in PLGA-MPs and the drug EE (%) were measured after extraction of drug from the microparticles. In order to extract DOX encapsulated in PLGA-MPs; DOX loaded and empty microparticles were weighted in triplicate (1 or 2 mg) and dissolved in 1 ml DMSO. Samples were vortexed and sonicated until the solution became clear. Each sample was divided into 200 µl aliquots and put in each well of a 96 well plate. Absorption of the solution was measured in Elisa Plate Reader at 480 nm. Empty-MPs were used a blank. Standards were prepared from known concentrations of DOX in DMSO. Once the amount of drug was calculated equation 1 was used to calculate EE, and equation 2 was used to calculate the theoretical drug loading (TL, %).

$$\text{Equation 1: EE(Encapsulation Efficiency,\%)} = \frac{\text{Amount of drug inside MPs (mg)}}{\text{Amount of drug initially used (mg)}} \times 100$$

$$\text{Equation 2: TL(Theoretical Drug loading,\%)} = \frac{\text{Amount of drug (mg)}}{\text{Amount of drug (mg) + Amount of PLGA(mg)}} \times 100$$

Characterization of PLGA coated microparticles

The sizes of empty microparticles and DOX loaded microparticles morphological properties were observed through SEM analyses. The hydrodynamic sizes and zeta potential were also determined with DLS measurements.

Drug release

Assessment of drug release from PLGA microspheres was done. DOX loaded microspheres, 10 mg for each method in duplicates, were placed into 2 ml Eppendorf tubes containing 1 ml phosphate buffered saline (pH 7.4). Release was followed over a specified time. Samples were incubated in a horizontal shaker at 37°C, with continuous agitation at 200 rpm. Sampling was performed at predetermined time intervals (1 - 41 days) and isolation of the microspheres was achieved by centrifugation. The microsphere suspensions were centrifuged at 14,000 rpm for 15 min. The clear supernatant (1 ml) was withdrawn and replaced with 1 ml fresh release medium. Upon centrifugation, settled microspheres were resuspended by vortexing. Supernatant was kept at -20°C until analysis.

Visualization of drug internalization

In order to observe the drug internalization from the SE microparticles, confocal scanning electron microscopy analysis was performed. By using the emission wavelength of DOX, particles were detected easily without extra dying procedure. Cells were incubated on the coverslips inside the wells of a six-well plate. First, coverslips were plated into wells and 3 ml of medium containing cells were poured onto wells (400,000 – 500,000 cells/well). Cells were incubated to provide attachment onto coverslips for 24 h. Different amounts of drug were added into the wells and incubated for 1 hour, then washed three times with PBS and fixation was done with 70% ethanol for 10 min. Excess ethanol was removed by washing with PBS.

Cell proliferation assay

DOX-sensitive (MCF-7) and DOX-resistant (MCF-7/1000 nM DOX) human breast cancer cells were used for the cell studies (Unsoy et al., 2012; Khodadust et al., 2013). Cells were grown in culture flasks in RPMI/1640 culture medium supplemented with 10% FBS, and 1% gentamycin solution at 37 °C under 5% CO_2. The cells were subcultured 2~3 times per week with 0.25% trypsin–EDTA. Antiproliferative effects of highest dose of drug, drug containing microparticles and empty MPs on sensitive and resistant MCF-7 cells were evaluated by means of the Cell Proliferation Kit (Biological Industries) according to manufacturer's instructions. Assay was a colorimetric test based on the reduction tetrazolium salt, XTT to colored formazan products by mitochondria of live cells. In brief, cells were seeded to 96-well microtiter plates (Greiner) at a concentration of 5.0 $x10^4$ cells/well and incubated for 72 h in medium containing horizontal dilutions of microparticles. In each plate assay was performed with a column of blank medium control and a cell control coloumn. Then, XTT reagent was added and soluble product was measured at 500 nm with an Spectromax 340 96-well plate reader (Molecular Devices, USA).

Results

Morphology of microparticles and DOX loaded microparticles

The shapes of microparticles prepared by SE and DE methods were studied under light microscope and scanning electron microscope (SEM). Both of the imaging techniques revealed considerable differences in the shapes and surface characteristics of microparticles (Figs. 1 and 2).

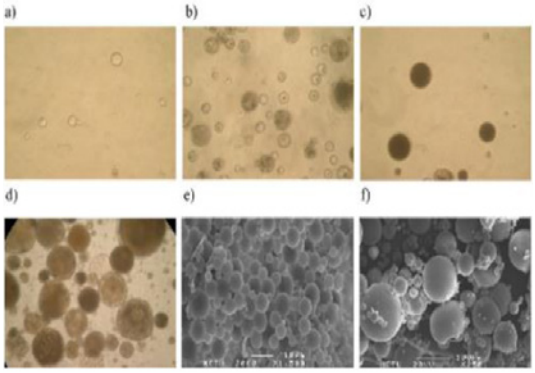


Figure 1. Morphology of DE microparticles**.** Light microscope images; a) 10 mg/ml PLGA, b) 30 mg/ml PLGA, c) 75 mg/ml PLGA, d) 100 mg/ml PLGA under 40X magnification. SEM images; e) 10 mg/ml PLGA, f) 75 mg/ml PLGA in the organic phase respectively.

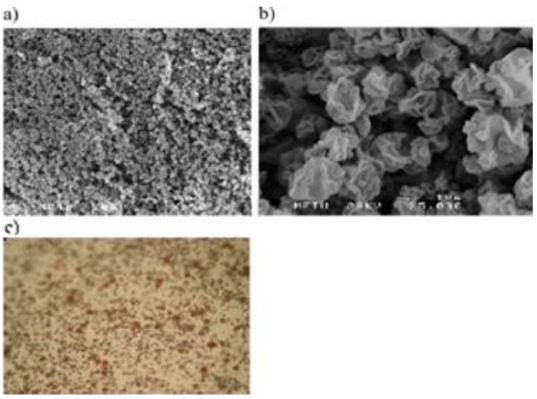
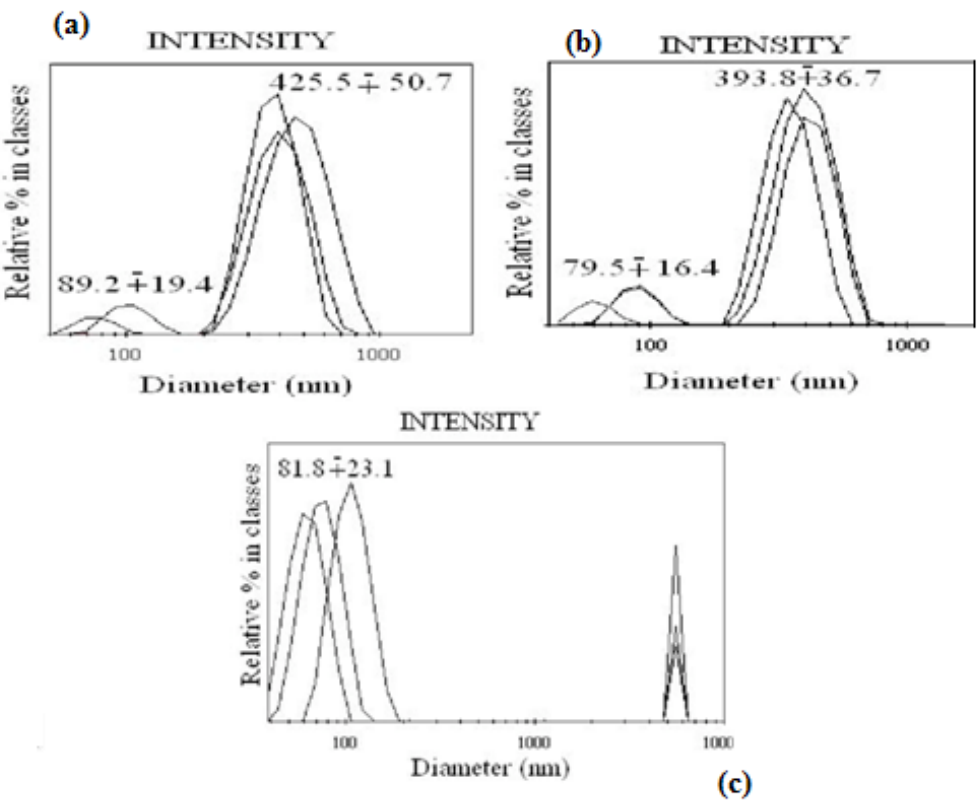


Figure 2. SEM images of DOX loaded SE microparticles. SEM images under a) 550X , b) 5000X c) light microscope image of SE particles (8 mg/ml PLGA) under 40X magnification.



PLGA (mg/ml)	Zeta (mV)	STDEV
10	-31.0	0.9
30	-28.8	3.0
75	-32.5	2.5

Figure 3. Size distribution graphs of DE (10mg/ml PLGA)(a), DE (30mg/ml PLGA)(b), DE (75mg/ml PLGA)(c) microparticle and zeta potential values.

Effects of polymer concentration and drug loading on EE on size and zeta potential

Figure 3 shows the effect of polymer concentration in the organic phase on the size distribution of particles prepared by DE solvent evaporation method (Tables 1 and 2).

DOX release from microparticles

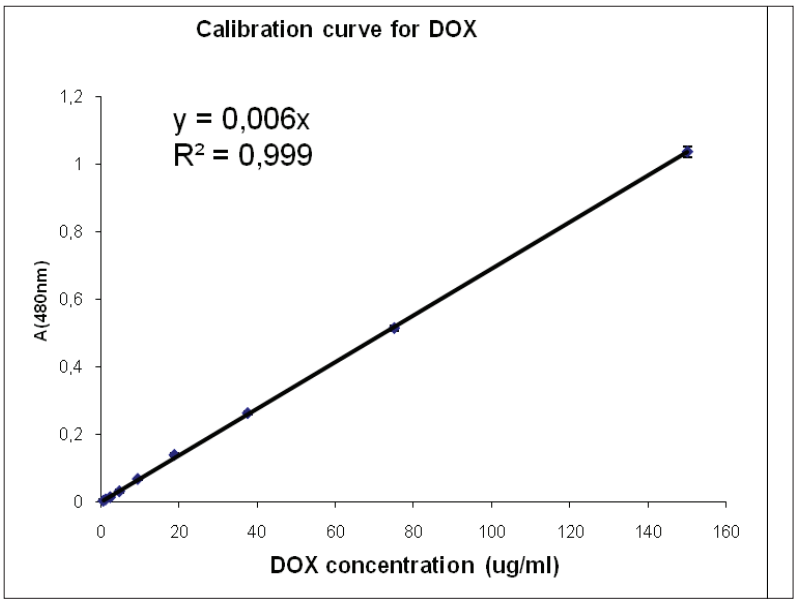
Figure 4a shows the standard curve of DOX in PBS. *In vitro* release of DOX from PLGA microparticles was followed over 40 days (Figure 4b). The release profiles from two different microparticle formulations were determined by reading the absorbance of released drug in phosphate buffer (PBS: 0.01 M, pH 7.4, 1ml) by using Elisa Reader at 480 nm. To measure DOX in PBS a new calibration curve was prepared.

Table 1. Different PLGA microparticle formulations and corresponding EE.

Sample	DOX(mg)	PLGA(mg/ml)	TL	EE(%)
DE-1	0.26	25	1	20.21
DE-2	0.52	25	2	14.92
DE-3	1.32	25	5	10.74
DE-4	0.53	10	1	15.12
DE-5	0.53	30	1	25.09
DE-6	0.53	75	1	40.00
SE-1	0.53	4	5	15.49
SE-2	1.11	4	10	45.69
SE-3	1.76	4	15	63.32
SE-4	2.5	4	20	71.53
SE-5	2.5	8	20	72.33

Table 2. Mean size and Zeta Potential of microparticles.

Sample	Mean size (nm)	Zeta (mV)
DE-4	(93%) 445.6±52.2	-31±0.98
DE-5	(90%) 393.8±25.9	-28.8±3.0
DE-6	(88%) 81.8±32.0	-32.5±2.54
SE-4	(100%) 474.7±18.0	-1.6±1.5
SE-5	(100%) 395.7±92.2	-10.5±0.28



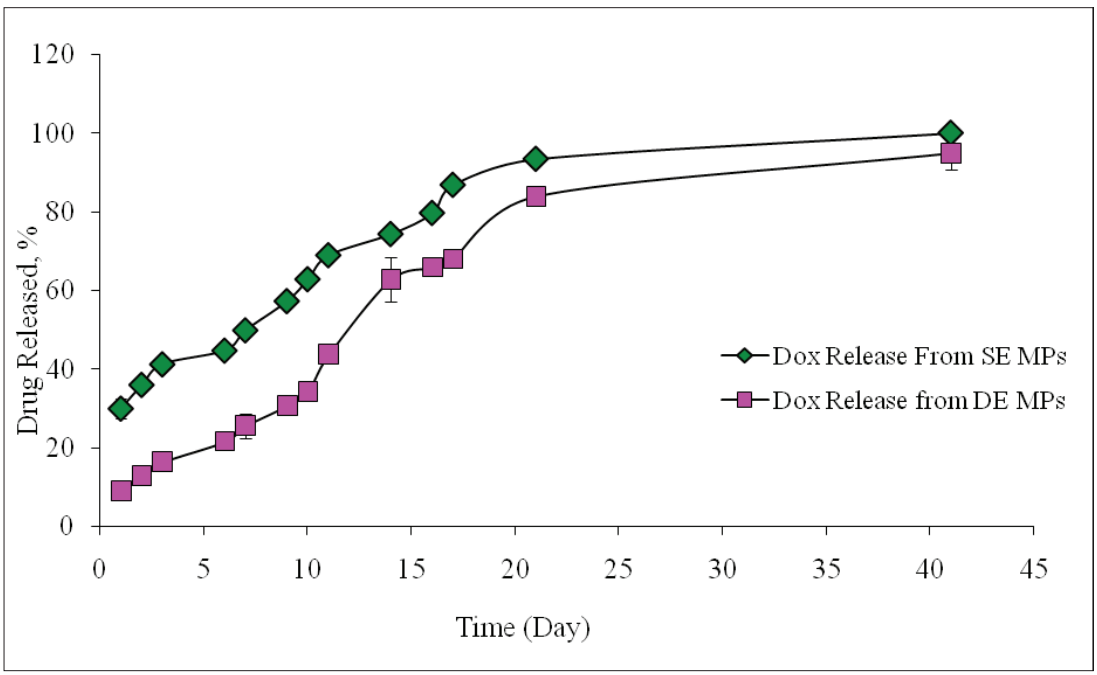


Figure 4. Calibration curve of DOX in PBS (a) and release profile of DOX from microparticles (b).

Internalization of DOX released from microparticles

Flourescence microscopy analysis results are given in Figure 5. MCF -7 cells incubated with free DOX showed the drug both in the prephery and cytoplasm. When drug is applied by means of microparticles it was observed that drug accumulation on the cell membrane increased. This might be the result of interactions of microparticles with the cell surface. This effect might aid internalization of the drug.

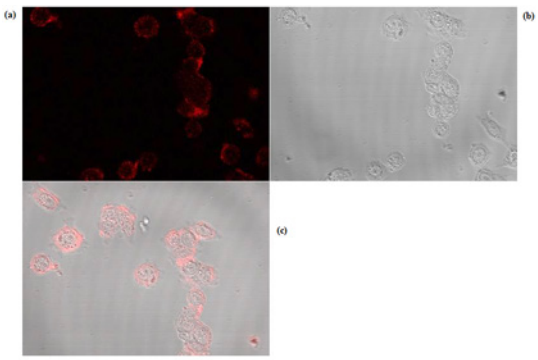
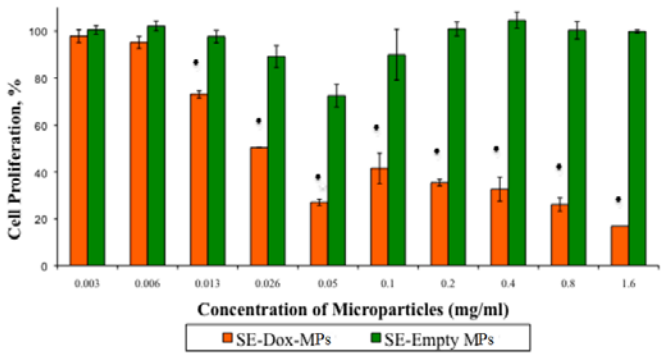


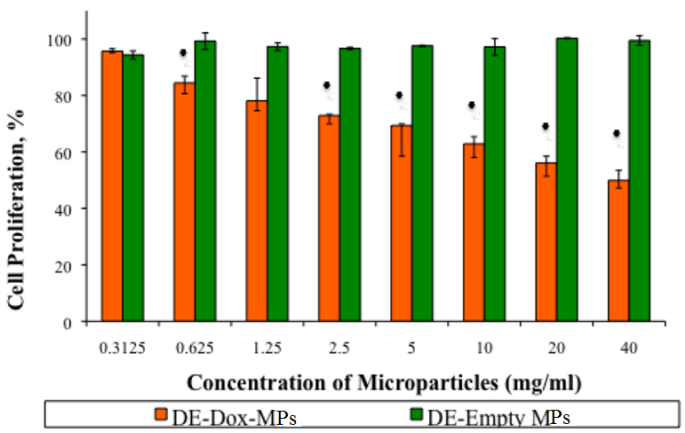
Figure 5. Confocal microscopy analysis MCF -7 incubated with free DOX**,** a), fluorescence image; b) light microscope image; c)superimposed image of a and b

Cell proliferation assay on PLGA microparticles

Figure 6 shows toxicity of encapsulated DOX on MCF-7 cells. Toxicity profile of SE-Dox-MPs suggested that concentration amounts less than 0.006 mg/ml caused a mild toxicity. Concentrations at 1.66 mg/ml killed almost %15 of all MCF-7 cells in the cell culture. To characterize the drug cytotoxicity the inhibition concentration (IC50) is used. It is given as the concentration of the drug concentration at which 50% cells stop metabolizing. After 72h of incubation, IC_{50} was determined as 0.095 mg/mL. This indicates the active release of doxorubicin is enough to elucidate cell killing effect even with very low dosages of particles. When compared to SE microparticles much more DE particles had be added to have 150 µM doxorubicin. That is due to their efficiency. After 72h of incubation, IC_{50} was determined as 30 mg/ml. IC_{50} for SE particles was 0.095 mg/ml. This means that 0.095 mg/ml of SE particles have the same cell killing effect as 30mg/ml DE particles.



(a)



(b)

Figure 6. Cytotoxic effect of empty and DOX loaded SE (a) and DE (b) microparticles on MCF-7 cells.

Discussion

Breast cancer is the most common form of cancer among women (Bertram 2000). DOX occupies a central position in the treatment of breast cancer. However it is associated with serious side effects such as cardio toxicity (Hanahan and Weinberg 2011). One approach to overcome DOX-related toxicity is to use polymeric drug carriers, which direct the DOX away from sites with tight capillary junctions such as the heart muscle, and allow usage of lower dosages. In the present study, DOX is encapsulated in biodegradable PLGA microparticles by w/o/w (water/oil/water) double emulsion (DE) and o/w (oil/water) single emulsion (SE) solvent evaporation techniques. The effects of several process factors on DOX entrapment efficiency (EE), particle size distribution, and zeta potential were examined.

Figure 1 and 2 show the light microscope and SEM images of MPs. When two fabrication methods were compared the most profound difference was among their sizes and surface properties. In general SE particles were much smaller than DE particles, however, there were very small DE particles as well. Comparison of size was also done by dynamic light scattering (Table 2). SE particles were red due to encapsulated DOX while DE particles were slightly pink. This is an expected result, since SE particles contain more drug than DE particles. A more detailed examination is done with SEM. DE particles all exhibited perfectly spherical shapes and smooth surfaces. There was pore formation to slight extent. There is very low affinity between PLGA solvent chloroform and non solvent water. This causes slow polymer precipitation. Slow precipitation is known to cause non porous dense particles with low initial release rate (Luan et al. 2006). The particles prepared by using SE method, however, were less spherical and had wrinkly surfaces. Moreover, the particle shape was not well defined. The appearance as deflated balls might have resulted due to lyophilization.

The maximum DOX entrapment efficiencies accomplished were 40.0 % and 72.3 % for DE and SE methods, respectively. Highest encapsulation efficiencies were accomplished by SE method. The low entrapment of DOX in DE formulation could be explained by preferential localization of drug at the outer aqueous phase rather than inside the microparticle core, which was less hydrophilic. In DE method drug and polymer were in different solvents that are immiscible with each other. Some drug partitioned into the aqueous medium during the emulsification and the evaporation of the organic solvent. In addition, some drug was lost in the repeated washings of the microspheres with water during the harvesting and extraction process. On the other hand in SE method, DOX was solubilized in chloroform which is immiscible with water, so its escape to the outer phase was minimized. In SE method, drug and polymer were dissolved in the same solvent and almost a homogenous solution is formed. Budhian et al. (2007) reported that higher drug polymer miscibility leads to higher drug incorporation. This might be the reason of higher drug contents achieved by SE formulation (Table 1). In DE method, the increase in theoretical drug loading from %1 to %5 resulted in a decrease of EE from %20.2 to %10.7. However, in SE method, EE increased at higher TL values.

Due to the limitations of DOX solubility in chloroform, PLGA concentration was increased from 4 mg/ml to a maximum of 8 mg/ml in particles prepared by SE method. This increase did not cause a significant change in size but it increased the zeta potential (Table 2). SE method produced particles with relatively lower zeta values. Zeta potential gives information about the stability of suspension, and high values are correlated with low aggregation of microparticles.

In Figure 3, the increase in polymer concentration from 10 mg/ml to 30 mg/ml resulted in limited change on the particle size. However, when the PLGA concentration is increased to 75mg/ml two peaks are formed one having smaller particles and another one having very large particles. Large particles might have resulted from poor polymer dispersion into water due to high viscosity of the organic phase. However they can be filtered away easily.

The release rate of DOX from SE particles was significantly higher than DE particles (Fig. 4). On day 1, the DOX microparticles prepared by SE method released 30± 2.5 % of

the total amount of encapsulated drug and the microparticles prepared by DE method released 3.6±0.2 %. About 50% of the total DOX was released by day 7 from the SE microspheres, and by day 13 from the DE constructs. SE and DE particles released about 90% of DOX by day 21 and 41 respectively. Tewes et al. (2007) showed that during the first hours of release DE particles released more drug than SE particles. They tried to explain this effect by stating that the superficial layers of microparticles formulated by SE were less enriched in DOX than those formulated by DE. The higher drug loading might be responsible for higher release. In general, the release increases with increasing loading in the case of water soluble drugs (Ravivarapu et al. 2000). Jiang et al. (2011) prepared PLGA microparticles loaded with doxorubin, PEGylated TNF-related apoptosis inducing ligand (PEGTRAIL), and with doxorubicin plus PEG-TRAIL using a w/o/w double emulsion (DE) solvent extraction method for effective tumor combination therapy. The encapsulation efficiency of 69.4 for doxorubicin and 87.7% for PEG-TRAIL was observed.

XTT assays showed that the drug is released from the microparticles and effective against resistant MCF-7 breast cancer cell line. In Figure 6, short-term cytotoxicity of microparticles on MCF-7 breast cancer cells are determined using XTT assay. There was a significant difference between the cytotoxicity of SE and DE microparticles. The lower EE and initial burst 3.6% of DE microparticles may have decreased its short-term cytotoxicity. Empty microparticles do not cause any cytotoxicity. Çetin et al. (2010) describes the preparation of anticancer drug-loaded poly(lactide-coglycolide) (PLGA) microparticles. PLGA microparticles loaded with Doxorubicin were prepared via o/w emulsion solvent evaporation. A cytotoxicity test was analysed by using Glioma RG2 cancer cells to investigate the cytotoxicity of DOX-loaded PLGA microparticles. DOX-loaded PLGA microparticles displayed a significant cytotoxicity toward the RG2 cells as compared to the unloaded PLGA microparticles.

Due to higher EE smaller amounts of SE-MPs are sufficient to exert the toxic effect. Even though microparticles were once hoped to overcome MDR (Barraud et al. 2005; Sahoo et al. 2005) some recent research failed to prove it. It is shown that P-gp substrates, such as DOX and paclitaxel, delivered to MDR cells by PLGA microparticle, are still susceptible to efflux by P-gp (Chavanpatil et al. 2006). Only a partial reversal of MDR seems to be obtained in this work from confocal microscopy of resistant MCF-7 cells treated with DOX entrapped MPs.

In summary, biodegradable polymeric microparticles were successfully produced by DE and SE emulsion solvent evaporation methods. The results demonstrated that the most appropriate method used to prepare DOX-loaded PLGA microparticles was the SE single emulsion technique. Processing parameters are optimized to obtain highest EE and optimum size distribution. Increasing the PLGA concentration leads to higher EE values in both of the methods investigated. Polymeric drug delivery systems can provide sustained release of drugs and direct drugs towards the tumor side so they are promising tools as alternative ways to overcome many challenges associated with conventional drug delivery.

References

Ambudkar S.V., Dey S., Hrycyna C.A., Ramachandra M., Pastan I., Gottesman M.M. (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annual Review of Pharmacology and Toxicology*, 39: 361-98.

Barraud L., Merle P., Soma E., Lefrançois L., Guerret S., Chevallier M., Dubernet C., Couvreur P., Trépo C., Vitvitski L. (2005) Increase of DOX sensitivity by DOX-loading into nanoparticles for hepatocellular carcinoma cells *in vitro* and *in vivo*. *Journal of Hepatology*, 42: 736-43.

Bertram J.S. (2000) The molecular biology of cancer. *Molecular Aspects of Medicine,* 21: 167-223.

Bilati U., Allémann E., Doelker E. (2005) Poly(D,L-lactide-co-glycolide) protein-loaded nanoparticles prepared by the double emulsion method--processing and formulation issues for enhanced entrapment efficiency. *Journal of Microencapsulation,* 22: 205-14.

Bilati U., Allémann E., Doelker E. (2005) Strategic approaches for overcoming peptide and protein instability within biodegradable nano- and microparticles. *European Journal of Pharmaceutıcs and Bıopharmaceutıcs,* 59: 375-88.

Budhian A., Siegel S.J., Winey K.I. (2007) Haloperidol-loaded PLGA nanoparticles: systematic study of particle size and drug content. *International Journal of Pharmaceutics,* 336: 367-75.

Cao N., Feng S.S. (2008) DOX conjugated to D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS): conjugation chemistry, characterization, *in vitro* and *in vivo* evaluation. *Biomaterials,* 29: 3856-65.

Cetin M., Vural I., Atila A., Kadıoğlu Y. (2010) Preparation and characterization of anticancer drug-loaded implantable PLGA microparticles. *Turk J Chem,* 34: 509 – 516.

Chavanpatil M.D., Patil Y., Panyam J. (2006) Susceptibility of nanoparticle-encapsulated paclitaxel to P-glycoprotein-mediated drug efflux. *International Journal of Pharmaceutics,* 320: 150-6.

Gabizon A., Catane R., Uziely B., Kaufman B., Safra T., Cohen R., Martin F., Huang A., Barenholz Y. (1994) Prolonged circulation time and enhanced accumulation in malignant exudates of DOX encapsulated in polyethylene-glycol coated liposomes. *Cancer Research,* 54: 987-92.

Hanahan D., Weinberg R.A. (2011) Hallmarks of cancer: the next generation.*Cell* 144: 646-74.

Iyer A.K., Khaled G., Fang J., Maeda H. (2006) Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today*, 11: 812-8.

Jabr-Milane L.S., van Vlerken L.E., Yadav S., Amiji M.M. (2008) Multi functional nanocarriers to overcome tumor drug resistance. *Cancer Treatment Reviews,* 34:592-602.

Jiang H.H., Kim T.H., Lee S., Chen X., Youn Y.S., Lee K.C.(2011) PEGylated TNF related apoptosis inducing ligand (TRAIL) for effective tumor combination therapy. *Biomaterials,* 32 (33): 8529–8537.

Khodadust R., Unsoy G., Yalçın S., Gündüz G., Gündüz U. (2013) PAMAM Dendrimer Coated Iron Oxide Nanoparticles: Synthesis and Characterization of Different Generations. *Journal of Nanoparticle Research,* 15: 1488

Luan X., Skupin M., Siepmann J., Bodmeier R. (2006) Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide-containing poly(lactide-co-glycolide) microparticles. *International Journal of Pharmaceutics,* 324: 168-75.

Phillips G.D.L., Li G., Dugger D.L., Crocker L.M., Parsons K.L., Mai E., Blättler W.A., Lambert J.M., Chari R.V., Lutz R.J., Wong W.L., Jacobson F.S., Koeppen H., Schwall R.H., Kenkare-Mitra S.R., Spencer S.D., Sliwkowski M.X. (2008) Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Research,* 68: 9280-90.

Ravivarapu H.B., Lee H., DeLuca P.P. (2000) Enhancing initial release of peptide from poly(d,l-lactide-co-glycolide) (PLGA) microspheres by addition of a porosigen and increasing drug load. *Pharmaceutıcal Development and Technology,* 5: 287-96.

Sahoo S.K., Panda A.K., Labhasetwar V. (2005) Characterization of porous PLGA/ PLA microparticles as a scaffold for three dimensional growth of breast cancer cells. *Biomacromolecules,* 6: 1132-9.

Takemura G., Fujiwara H. (2007) DOX-induced cardiomyopathy fromthe cardiotoxic mechanisms to management. *Progress in Cardıovascular Diseases*, 49: 330–52.

Tewes F., Munnier E., Antoon B., Ngaboni Okassa L., Cohen-Jonathan S., Marchais H., Douziech-Eyrolles L., Soucé M., Dubois P., Chourpa I. (2007) Comparative study of DOX-loaded poly(lactide-co-glycolide) nanoparticles prepared by single and double emulsion methods. *European Journal of Pharmaceutıcs and Bıopharmaceutıcs*, 66: 488-92.

Unsoy G., Yalçın S., Khodadust R., Gündüz G., Gündüz U. (2012) Synthesis optimization and characterization of chitosan coated iron oxide nanoparticles produced for biomedical applications. *Journal of Nanoparticle Research*, 14: 964.