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RESEARCH ARTICLE

Effect of Organic Solvents on Gemcitabine Loaded PLGA Nanoparticles

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SUMMARY

Gemcitabine hydrochloride is a water soluble drug and widely used alone or in combination with other chemotherapeutic agents for the treatment of breast cancer. In this study, gemcitabine hydrochloride loaded poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles (NPs) were prepared by modified double emulsion solvent evaporation method. PLGA is a polymer, used in many biomedical applications because it is biodegradable, biocompatible, and FDA approved. To investigate the effect of type of organic phase solvents on the mean particle sizes and entrapment efficiency of obtained PLGA nanoparticles, different organic solvents acetone (ACE, water-soluble solvent) and dichloromethane (DCM, water-immiscible solvent) were used as organic solvents either individually or in combinations. When DCM used alone as organic solvents, large NPs above 400 nm were obtained. DCM in combination with ACE (DCM:ACE 1:2, v/v) resulted in highest entrapment efficiency. The optimized formulation had a range of 231 ± 10.3 nm particle sizes and 12.0 ± 0.43% entrapment efficiency. These findings show that the solubility of organic phase solvents in water was an important parameter affecting the mean particle size and entrapment efficiency of gemcitabine hydrochloride loaded PLGA nanoparticles.

Key Words: *Gemcitabine, modified double emulsion solvent evaporation method, PLGA, nanoparticles*

Gemsitabin Yüklü PLGA Nanopartiküllerine Organik Çözücü Etkisi

ÖZET

Gemsitabin hidroklorür meme kanseri tedavisinde tek başına veya diğer kemoterapötiklerle kombine halde sıklıkla kullanılan suda çözünür bir ilaçtır. Bu çalışmada gemsitabin hidroklorür yüklü poli (d,l-laktid-ko-glikolid) (PLGA) nanopartiküllerin hazırlanmasında modifiye çift emülsiyon çözücü uçurma yöntemi kullanılmıştır. PLGA birçok biomedikal uygulamada kullanılan, FDA'den onay almış, biyoparçalanabilir ve biyouyumlu bir polimerdir. Organik çözücünün partikül büyüklüğü ve yükleme kapasitesi üzerindeki etkisinin incelenmesi için su ile karışabilen aseton ve su ile karışmayan diklorometan tek tek ve karışımları halinde kullanılmıştır. Sadece diklorometan kullanıldığında partikül büyüklüğü 400 nm'den fazla olan nanopartiküller elde edilmiştir. Diklorometan : aseton (1:2, h/h) karışımı kullanıldığında en yüksek yükleme verimliliği elde edilmiştir. Optimize edilmiş formülasyon 231 ± 10.3 nm partikül büyüklüğüne ve % 12.0 ± 0.43 yükleme verimliliğine sahiptir. Bulgular kullanılan organik çözücünün su ile karışabilirliğinin gemsitabin hidroklorür yüklü nanopartiküllerin partikül boyutunu ve yükleme kapasitesini etkilediğini göstermiştir.

Anahtar Kelimeler: *Gemsitabin, modifiye çift emülsiyon çözücü uçurma yöntemi, PLGA, nanopartikül*

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INTRODUCTION

Gemcitabine hydrochloride, which is a difluoro analog of deoxycytidine and an anti-metabolite, is currently used in clinics for the treatment of several types of human cancers including breast, ovarian, and non-small cell lung and metastatic pancreatic cancers (Derakhshandeh, 2012; Abbruzzese, 1991). Gemcitabine hydrochloride, which has a 1.4 log P value, is a highly hydrophilic molecule (Trickler, 2010). Formulation of hydrophilic compounds in nanoparticles is problematic due to their escape to the external aqueous phase (Cohen-Sela, 2009). Different nanocarriers such as nanospheres, nanocapsules, liposomes, micelles, dendrimers, quantum dots, solid lipid nanoparticles, polymeric nanoparticles, gold nanoparticles, virus and virus-like nanoparticles have been explored for the delivery of small molecules and large molecules therapeutics (Vaze, 2016). Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties. The polymeric nanoparticles are prepared from biocompatible and biodegradable polymers in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. The performance of the nanoparticles depends on several factors such as polymer nature (polymer molecular weight and copolymer composition), organic solvents, type, and concentration of stabilizer, etc. Among these factors, the selection of organic solvent is critical in developing a successful nanoparticulate formulation (Sahana, 2008). The aim of this study was to prepare PLGA nanoparticles containing highly hydrophilic drug to achieve better entrapment efficiency. This study describes the effect of various organic solvents including water-immiscible solvent, and fully water-soluble solvent on the particle size of PLGA nanoparticles prepared by the modified double emulsion solvent evaporation method.

MATERIALS AND METHODS

Materials

PLGA (lactide:glycolide ratio of 50:50, acid terminal groups, 7–17 kDa) and poly(vinyl alcohol) (PVA, MW 31-50 kDa, 87-89% hydrolyzed) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Gemcitabine hydrochloride was a gift from Koçak Farma (Turkey). The organic solvents, dichloromethane (DCM), acetone (ACE) and acetonitrile were also purchased from Sigma–Aldrich. The water used in the experiments was deionized and filtered (Mes UP1104 Barnstead, USA). All chemicals were either extra pure or chromatography grade.

Preparation of gemcitabine hydrochloride loaded NPs

The nanoparticles containing gemcitabine hydrochloride were prepared using modified double emulsion solvent evaporation method (Mondalek, 2008; Shi, 2013) as shown in Figure 1. Gemcitabine hydrochloride (2 mg) was dissolved in aqueous solution (1.6 mL) of PVA to form aqueous phase which was then added to a solution of 240 mg PLGA in 4 mL of organic solvent/solvent system to give a w/o emulsion. ACE and DCM were employed as organic solvents either individually or in combinations. This primary emulsion was sonicated for 60 s at 20W (Sonics-VCX 130 FSJ, USA) over an ice bath and added to external aqueous solution (6 mL) of PVA to form multiple emulsion. The multiple emulsion was

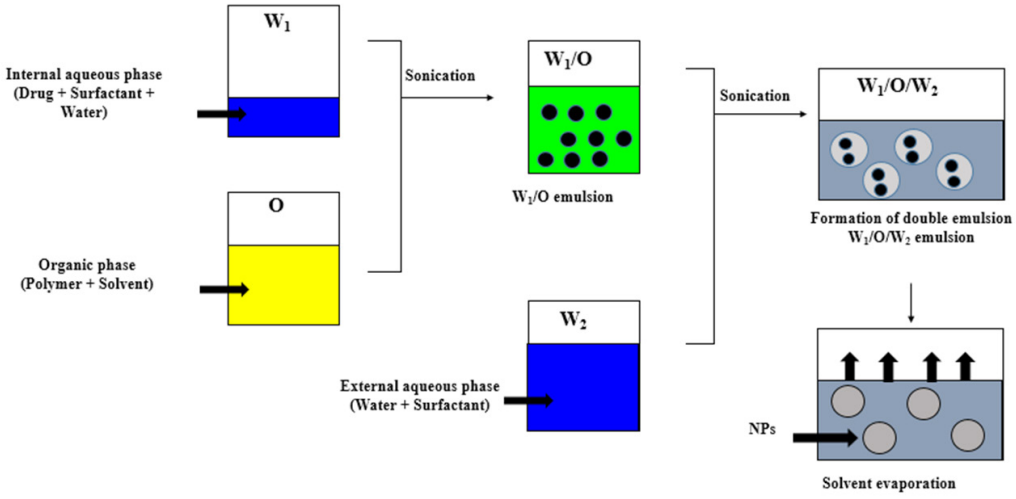

Figure 1. Preparation of gemcitabine hydrochloride loaded PLGA nanoparticles

again sonicated for 60 s to reduce the particle size. After evaporation of organic phase under reduced pressure in a rotary evaporator (Rotavapor® R-3, Büchi, Switzerland), nanoparticles were recovered by centrifugation (Allegra X-30R Beckman Coulter, Germany) at 18.000 rpm for 45 min.

As a stabilizer, PVA was utilized and dissolved in both the inner and external aqueous phases 1% w/v and 2% w/v, respectively. The composition of gemcitabine hydrochloride loaded PLGA nanoparticles are given in Table 1.

Freeze-drying of nanoparticle dispersion

Freshly prepared nanoparticle dispersions were freeze-dried in aliquots of 1.5 mL in 10 mL glass vials. The vials were frozen at −80°C for 30 min. The frozen samples were lyophilized for 40 h at -55°C (Christ Alpha 1-2 LD plus, Germany). Mannitol (10% w/w) was selected as cryoprotectant.

Table 1. The composition of gemcitabine hydrochloride loaded PLGA nanoparticles

	Formulation code				
Organic phase (4mL)	**NP1**	**NP2**	**NP3**	**NP4**	**NP5**
PLGA 50:50 7-17 kDa (mg)	240	240	240	240	240
DCM (mL)	4	-	-	-	-
ACE (mL)	-	4	-	-	-
DCM:ACE (1:1, v/v) (mL)	-	-	4	-	-
DCM:ACE (1:2, v/v) (mL)	-	-	-	4	-
DCM:ACE (1:4, v/v) (mL)	-	-	-	-	4
Inner aqueous phase (1.6 mL)					
Gemcitabine hydrochloride (mg)	2	2	2	2	2
PVA (31-50 kDa) (%, w/v)	1	1	1	1	1
External aqueous phase (6 mL)					
PVA (31-50 kDa) (%, w/v)	2	2	2	2	2

Characterization of nanoparticles

Particle size and zeta potential

The particle size, polydispersity index and zeta potential of the nanoparticles were determined using a Zetasizer Nano ZS (Malvern Instruments, UK). For this, freeze-dried nanoparticles were dispersed in distillated water and sonicated for 5 min and then the measurements were taken in triplicate at 25° C.

Entrapment efficiency (EE%)

Freeze-dried nanoparticles (5 mg) were weighed, and then mixed with 5 mL of DCM and vortex-mixed. This mixture was sonicated in an ultrasonic bath and 10 mL of phosphate buffer solution (pH 7.4) was added into it and mixed for 60 min. Then, the remaining aqueous dispersion was filtered and samples were analyzed by HPLC at 268 nm. The values are averages of three replicates (n = 3).

The following equation was used to evaluate the entrapment efficiency (EE%):

$$EE\% = \frac{D_{NP}}{D_T} \times 100 \quad (1)$$

where D_{NP} is the amount of the drug in NPs and D_T is the total amount of the drug added.

HPLC method: A reverse phase C 18 column (250 mm x 4.6 m, 5 µm particle size, Waters Xselect, Ireland) was used with HPLC-UV visible detector. The mobile phase was water/acetonitrile (95:5 v/v). The flow rate was 1 mL/min and the UV detection was performed at 268 nm.

Statistical analysis

All data were expressed in the form of the mean ± standard deviation. The difference between two parameters were considered statistically significant for $p<0.05$. All the analysis of data was performed using statistical software package Graphpad Prism 5. Statistical analyses were done using one-way ANOVA with the Tukey post hoc test.

RESULTS AND DISCUSSION

Effect of organic solvent type and ratio

In this study, it was observed that the entrapment efficiency and particle size were effected by the organic solvent. However, the ratio of organic solvent mixture (DCM:ACE) did not affect the particle size significantly ($p>0.05$), while entrapment efficiency was significantly influenced ($p<0.05$) as shown in Figure 2. The properties of gemcitabine hydrochloride loaded PLGA nanoparticles prepared by utilizing different

solvents/solvent ratios were summarized in Table 2. When DCM was used as the organic phase in NP1 formulation, significant aggregation was obtained and the particle size value was found to be above 400 nm due to immiscible properties of DCM. It is well known that ACE is miscible with water and it is partitioned and diffused into water. On the other hand, stable primary emulsion was not formed with the use of ACE because of the miscibility with water (NP2). Similar result has also been reported by Sahana et al (Sahana, 2008). Different DCM:ACE ratios (1:1, 1:2 and 1:4, v/v) were also investigated. The highest entrapment efficiency was achieved when DCM:ACE (1:2, v/v) solvent mixture was used (NP4). This result can be explained by water miscibility of ACE that diffuses quickly to the external aqueous phase and this constitutes local supersaturation areas near the interface, which induce polymer precipitation and solidification when the critical concentration is attained. This could prevent the rapid escape of hydrophilic drug content to the external aqueous phase. On the other hand, a low entrapment efficiency was observed in DCM:ACE (1:4, v/v) due to the high level of ACE (NP5). Ruan et al and Manoochehri et al also reported that there was an optimized mixing ratio of DCM and ACE to yield high EE (Ruan, 2002; Manoochehri, 2013). Manoochehri et al used the ACE: DCM ratio from 10:90 to 40:60 and they reported that entrapment efficiency showed the same trend and a decrease in entrapment efficiency was observed with increasing ACE to DCM ratio. The effect of DCM:ACE mixture ratio was also investigated on particle size. When the ACE ratio increased, particle sizes were found almost similar in all mixtures ($p>0.05$). Regarding zeta potential, all nanoparticle formulations displayed a negative surface charge. The polydispersity index (PDI) of the NPs showed values from 0.130 ± 0.026 to 0.342 ± 0.156, indicating homogenous populations (PDI < 0.4) of NPs.

Table 2. Effects of organic solvent on the particle characteristics of gemcitabine hydrochloride loaded NPs (n=3)

Formulation Code	Organic Solvent	Particle size (nm)	PDI	Zeta (mV)	EE %
NP1	DCM	486±24.4*	0.342±0.156	-18.4±2.44	4.87±0.07
NP2	ACE	246±8.93	0.221±0.048	-21.7±0.61	1.67±0.01
NP3	DCM:ACE (1:1, v/v)	213±3.00	0.130±0.026	-11.6±0.26	6.92±0.48
NP4	DCM:ACE (1:2, v/v)	231±10.3	0.225±0.040	-19.2±1.61	12.0±0.43
NP5	DCM:ACE (1:4, v/v)	237±16.6	0.270±0.047	-14.0±1.94	4.21±0.15

* represents a significant difference of NPs between NP1 and other formulations ($p<0.05$)

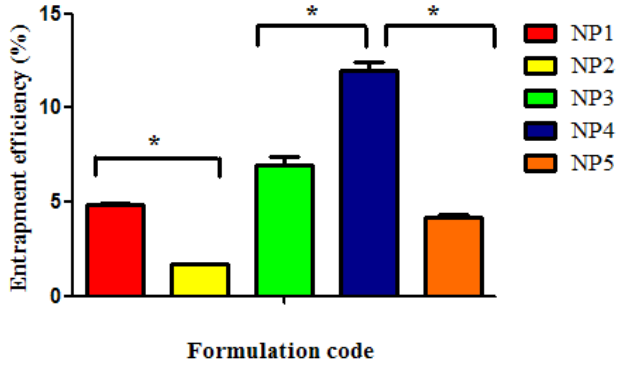

Figure 2. Effect of organic solvents/ solvent systems on entrapment efficiency

* represents a significant difference between the formulations ($p<0.05$)

CONCLUSION

Gemcitabine hydrochloride loaded PLGA nanoparticles can be prepared successfully by modified double emulsion solvent evaporation method. The present study suggests that organic solvents play a crucial role in nanoparticle formulation. Physical properties of the organic solvents strongly effect the particle size and entrapment efficiency. For further studies, different molecular weight and copolymer compositions of PLGA can be investigated to increase entrapment efficiency of hydrophilic drug and to obtain small particle size.

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REFERENCES

Abbruzzese, J.L., Grunewald, R., Weeks, E.A., Gravel, D., Adams, T., Nowak, B., Mineishi, S., Tarassoff, P., Satterlee, W., Raber, M.N. (1991), A phase I clinical, plasma, and cellular pharmacology study of gemcitabine, J Clin Oncol, 9(3), 491-498.

Cohen-Sela, E., Teitlboim, S., Chorny, M., Koroukhov, N., Danenberg, H.D., Gao, J., Golomb, G. (2009), Single and double emulsion manufacturing techniques of an amphiphilic drug in PLGA nanoparticles: formulations of mithramycin and bioactivity, J Pharm Sci, 98(4), 1452-1462.

Derakhshandeh, K., Fathi, S. (2012), Role of chitosan nanoparticles in the oral absorption of Gemcitabine, Int J Pharm, 437, 172-177.

Manoochehri, S., Darvishi, B., Kamalinia, G., Amini, M., Fallah, M., Ostad, S.N., Atyabi, F., Dinarvand, R. (2013), Surface modification of PLGA nanoparticles via human serum albumin conjugation for controlled delivery of docetaxel, Daru, 21(1), 1-10.

Mondalek, F.G., Lawrence, B.J., Kropp, B.P., Grady, B.P., Fung, K.M., Madihally, S.V., Lin, H.K. (2008), The incorporation of poly(lactic-co-glycolic) acid nanoparticles into porcine small intestinal submucosa biomaterials, Biomaterials, 29(9), 1159-66.

Ruan, G., Feng, S.S., Li, Q.T. (2002), Effects of material hydrophobicity on physical properties of polymeric microspheres formed by double emulsion process, J Control Release, 84(3), 151-160.

Sahana, D.K., Mittal, G., Bhardwaj, V., Kumar, M.N. (2008), PLGA nanoparticles for oral delivery of hydrophobic drugs: influence of organic solvent on nanoparticle formation and release behavior in vitro and in vivo using estradiol as a model drug, J Pharm Sci, 97(4), 1530-1542.

Shi, L., Wang, X., Zhao, F., Luan, H., Tu, Q., Huang, Z., Wang, H., Wang, H. (2013), In vitro evaluation of 5-aminolevulinic acid (ALA) loaded PLGA nanoparticles, Int J Nanomedicine, 8, 2669-2676.

Trickler, W.J., Khurana, J., Nagvekar, A.A., Dash, A.K. (2010), Chitosan and glyceryl monooleate nanostructures containing gemcitabine: potential delivery system for pancreatic cancer treatment, AAPS PharmSciTech, 11, 392-401.

Vaze, O. S. (2016), Pharmaceutical Nanocarriers (Liposomes and Micelles) in Cancer Therapy, J Nanomed Nanotechnol, 7(3), 1-2.