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*RESEARCH ARTICLE*

# Liposomes Containing Imatinib Mesylate and Dexketoprofen Trometamol: Development and Characterization

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***Liposomes containing imatinib mesylate and dexketoprofen trometamol: development and characterization***

***SUMMARY***

*Imatinib mesylate (IMA) is a tyrosine kinase receptor inhibitor and its anticancer efficiency hold on inhibition of Bcr-Abl pathway. Dexketoprofen trometamol (DEX) is a non-sterodial anti-inflammatory drug and demonstrated its anticancer efficiency on particularly animals with colorectal cancer in recent studies. Therefore it is aimed both decreasing the pain which is appeared during cancer therapy and reducing tumor size. IMA and DEX were co-loaded into DPPC/Cholesterol /DSPE-PEG2000 liposomes using different methods of production. The formulations were evaluated in terms of zeta potential, particle size distribution, polydispersity index, encapsulation efficiency and in vitro release profile and the optimal formulations were determined. As a result of studies we have obtained liposomes, which have low particle size, high zeta potential with negative charge, and high entrapment efficiency for both of drugs.*

***Key Words:*** *Liposome, imatinib mesylate, drug combination, cancer*

***İmatinib mesilat ve deksketoprofen trometamol içeren lipozomlar: geliştirilmesi ve karakterize edilmesi***

***ÖZET***

*İmatinib mesilat (IMA), tirosin kinaz reseptör inhibitörüdür ve Bcr-Alb yolağını inhibe ederek antikanser etkinlik göstermektedir. Deksketoprofen trometamol (DEX) ise non-steroidal antiinflamatuvar bir ilaç olup, son zamanlarda yapılan çalışmalar sonucunda özellikle kolorektal kanserli hayvanlar üzerinde antikanser etkinlik gösterdiği gözlenmiştir. Bu nedenle bu çalışma ile kanser tedavisi sırasında gözlenen ağrıyı azaltmak ve tümör boyutunu küçültmek hedeflenmiştir. Farklı üretim yöntemleri kullanılarak DPPC/kolesterol/DSPE-PEG2000'den oluşan lipozomların içine IMA ve DEX maddeleri yüklenmiştir. Geliştirilen formülasyonlar zeta potansiyeli, partikül büyüklük dağılımı, polidispersite indeksi, etkin madde yükleme verimililiği ve in vitro ilaç salım profillerine gore değerlendirilmiş ve en uygun formülasyon belirlenmiştir. Çalışma sonuçlarına gore düşük partikül büyüklüğüne, yüksek zeta potansiyeline ve her iki ilaç için yüksek yükleme verimliliğine sahip lipozomlar elde edilmiştir.*

***Anahtar kelimeler:*** *Lipozom, imatinib mesilat, ilaç kombinasyonu, kanser*

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## INTRODUCTION

The cancer is a disease which effects quality of millions people's lives and negatively economy worldwide. According to American Cancer Society's reports, even though death rate from cancer in the US has declined over the past two decades, in 2016 1,685,210 new cancer cases and 595,690 deaths from cancer are occurred (Simon, 2016). Therefore, the cancer therapy is still the focus of attention of researchers and maintain up to date.

Imatinib mesylate (IMA) is a potent and highly selective tyrosine kinase inhibitor and located in the drug market with Gleevec brand name, which has been approvaled as a standard treatment for chronic myeloid leukemia (CML) and gastrointestinal stromal tumor (GIST) by FDA in 2001 (Pinto, 2011). Main molecular targets of imatinib include Bcr/Abl in CML, c-kit in GIST and platelet derived growth factor receptor (PDGFR) in several malignancies, including prostate cancer (Buchdunger, 2002). Imatinib prevents PDGFR activation and subsequent signaling pathways responsible for cell growth, survival, metastases, andangiogenesis (Ostman, 2007).

Dexketoprofen trometamol (DEX) is a soluble salt of dextrorotatory enantiomer of non-steroidal anti-inflammatory drug ketoprofen. Racemic ketoprofen is used as an analgesic and anti-inflammatory agent and inhibits prostaglandin synthesis though inhibition of COX-1 and COX-2 enzymes (Miranda, 2011). Prostaglandin synthesis increases in some cancer types. Therefore recently, attention on NSAIDs have increased due to their potential as chemotherapeutic agents against cancer (Khuder, 2001; Senthilraja, 2009)

Liposomes have uniqe properties due to their bilayer structure like human cell membranes. Their phospholipids and cholesterol ingredients make them biocompatible with biological membranes and results that were obtained from Caco-2 cells studies support that it is biocompatible and non-immunogenic (Yücel, 2010; Pham, 2014). Nowadays, drug-delivery systems are frequently used in cancer treatments for providing effective targeted therapies by binding a ligand to the surface.

Liposomes have some advantages and disadvantages. Lipophilic and hydrophilic drugs are loaded into liposomes in consequence of their bilayer amphiphilic structure. On the other hand, oxidation of phospholipid, seen as a stability problem in liposomes (Pham, 2014).

In this study, several articles were utilized (Mendonça, 2010; Özçetin, 2011) and liposome are prepared using different methods. These liposomes were specified optimal formulations which are evaluated in terms of entrapment efficiency (EE), zeta potential (ZP), particle size distribution (PSD), polydispersity index (PI) and in vitro release profile.

## MATERIALS AND METHODS

### Materials

Dipalmitoylphosphatidylcholine (DPPC) and methoxy-poly(ethyleneglycol)2000-distearoylphosph atidylethanolamine (DSPE-$PEG_{2000}$) were purchased from Avanti Polar Lipids, USA and cholesterol was also purchased from Sigma, USA. Methanol and chloroform were from Sigma, Germany. Imatinib mesylate were from Biotang, USA. Dexketoprofen trometamol was provided by Nobel Pharma Ltd, Turkey as a gift sample. All chemicals were analytical grade and were used without further purification.

### Preparation of imatinib mesylate and dexketoprofen trometamol loaded liposome

The liposomes were prepared through different methods. According to Method 1 (Liposome-A), DPPC, cholesterol and DSPE-$PEG_{2000}$ (1:1:0.1 w/w) were dissolved in methanol:chloroform (1:1 v/v) in round bottom flask and the organic solvent was removed at a temperature above phase transition temperature ($T_m$) of the lipids under reduced pressure using rotary evaporator. After a dry homogeneous film was formed, the film was hydrated with distilled water using magnetic stirring at room temperature for 1 h followed by sonicated for 1 h at 45°C. IMA and DEX were dispersed in 0.5 M NaCl containing pH 7.4 phosphate buffer and then added to the liposome suspension. Liposome suspension containing IMA and DEX was incubated at 60°C for 1 h to load into liposome. According to Method 2 (Liposome-B and Liposome-C), DPPC, cholesterol and DSPE-$PEG_{2000}$ (1:1:0.1 w/w) were dissolved in methanol:chloroform (1:1 v/v) in round bottom flask and the organic solvent

was removed at a temperature above phase transition temperature ($T_m$) of the lipids under reduced pressure using rotary evaporator. After a dry homogeneous film was formed, the film was hydrated with distilled water containing IMA and DEX and the system was sonicated by probe type sonication for 8 minutes (30 sec sonication followed by 30 sec rest, Amplitude %20) and then vortexed for 5 minutes followed by sonicated again for 8 minutes. According to last Method 3 (Liposome-D), DPPC, cholesterol and DSPE-$PEG_{2000}$ (1:1:0.1 w/w) were dissolved in methanol:ethylacetate (0.5:4.5 v/v) in round bottom flask and half of the IMA containing pH 7.4 phosphate buffer was added to flask. Then the system was sonicated for 15 minutes and the organic solvent was removed at 35-37°C under reduced pressure using rotary evaporator. After a dry homogeneous film was formed, the film was hydrated with remaning IMA containing pH 7.4 phosphate buffer using magnetic stirring at room temperature for about 1 h. Informations related to studies were summarized in Table 1.

**Table 1.** The formulations containing IMA and DEX

| Formulation Code | Liposome-A | Liposome-B | Liposome-C | Liposome-D |
|---|---|---|---|---|
| **Cholesterol** | 10 mg | 10 mg | 10 mg | 10 mg |
| **DPPC** | 10 mg | 10 mg | 10 mg | 10 mg |
| **DSPE-$PEG_{2000}$** | 1 mg | 1 mg | 1 mg | 1 mg |
| **IMA** | 10 mg | 10 mg | 10 mg | 10 mg |
| **DEX** | 3 mg | 10 mg | 3 mg | X |
| **MeOH:Choloroform** | 1:1/5 mL | 1:1/5 mL | 1:1/5 mL | X |
| **MeOH:Etyhlacetate** | X | X | X | 0.5:4.5/5 mL |
| **First Hdyration** | Distilled water/10 mL | Distilled water/2 mL | Distilled water/20 mL | pH 7.4 phosphate buffer/2 mL |
| **Second Hdyration** | 0.5 M NaCl containing pH 7.4 phosphate buffer/10 mL | X | X | X |
| **Method** | Incubation | Probe sonication | | Magnetic stirring |

**Evaluation of imatinib mesylate and dexketoprofen trometamol loaded liposomes**

Prepared drug delivery systems were centrifuged and drug substances (IMA and DEX) in supernatant was analyzed by using UPLC (Ultra Performance Liquid Chromatography) method in order to get EE of formulations. ZP, PSD and PI were determined by Malvern Zetasizer Nanoseries. *In vitro* release studies were performed in pH 7.4 phosphate buffer (n=3).

**Entrapment efficiency of liposome**

Entrapment efficiency of IMA and DEX within prepared drug delivery systems was determined by centrifuging 25000 g for 20 min at 4°C and drug substances (IMA and DEX) in supernatant were analyzed by using UPLC (Ultra Performance Liquid Chromatography) method. Entrapment efficiency was calculated based on the ratio of amount of drug present in the drug delivery systems to amount of drug used in loading process.

EE(%)=(Total amount of IMA or DEX in the liposome)/( Initial amount of IMA or DEX for loading studies ) x 100 (1)

***In vitro* drug release studies**

*In vitro* drug release profile of IMA and DEX from drug loaded liposome were done by Franz diffusion cell test method for 72 h at pH 7.4 phosphate buffer. A known quantity of IMA and DEX loaded liposomes were dispersed in 1.5 ml distilled water as a test sample. 0.048 mg of IMA and 0.09 mg of DEX were dissolved in 1.5 ml distilled water as a reference sample. Test and reference samples were separately placed on the upper donor chamber of Franz cells and receptor chamber of Franz cells were filled with 2.5 ml phosphate buffer at pH 7.4. Franz cells were then incubated in a water bath shaker at 37°C. At certain time intervals, aliquots were withdrawn from cells and replenished with freshly prepared buffer. The cumulative percentage of release was calculated and the mean values and standard deviation were reported.

## RESULTS

The prepared formulations using different methods and materials were evaluated with regards to particle size distribution, polydispersity index, zeta potential and entrapment efficiency and the results were summarized in Table 2. When the results were taken into account, optimal formulation for characteristic properties such PSD, PI and DEX entrapment efficiency was Liposome-B. Therefore, *in vitro* drug release studies were performed for this formulation. The results of drug release studies were shown in Figure 1 and 2.

**Table 2.** The results in regard to formulations

| Formulation Code | | Liposome-A | Liposome-B | Liposome-C | Liposome-D |
|---|---|---|---|---|---|
| Entrapment efficiency | IMA | 99,74% | 97,46% | 38,72% | - |
| | DEX | 3,64% | 61,92% | 34,41% | X |
| Particle Size Distribution (nm) | | 240,6±102,2 | 347,1±80,0 | 320,4±95,4 | 418,5±27,4 |
| Polydispersity Index | | 0,471±0,252 | 0,584±0,140 | 0,613±0,245 | 0,646±0,343 |
| Zeta Potential (mV) | | -12,5±5,2 | -9,9±1,6 | -12,8±2,3 | -17,5±0,9 |

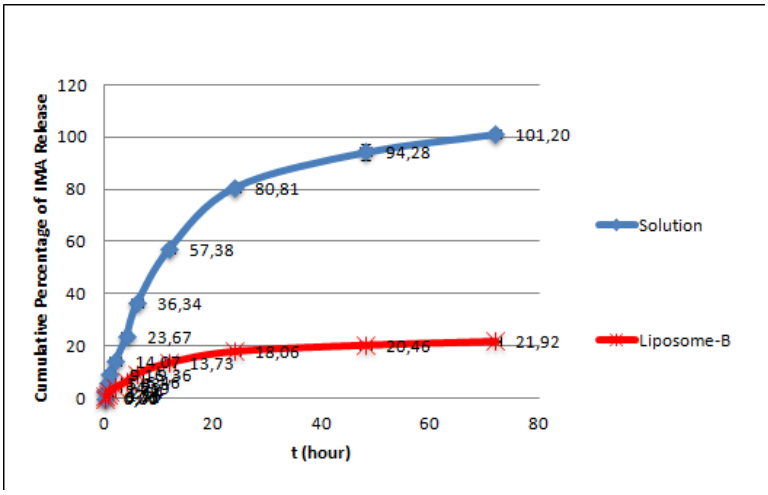


**Figure 1.** *In-vitro* drug release of IMA from liposome and solution

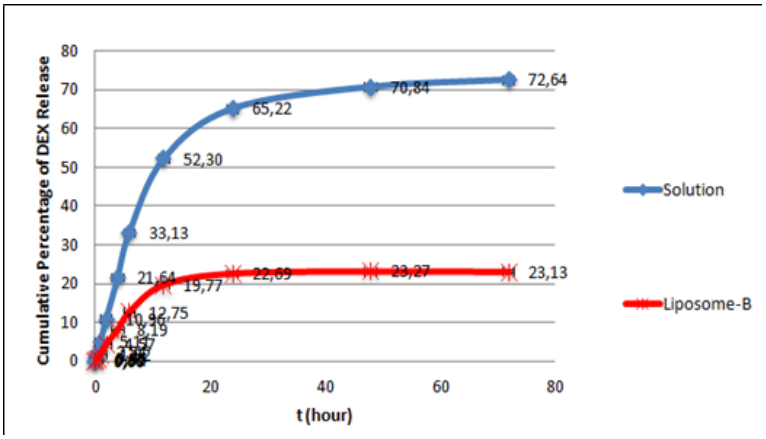


**Figure 2.** *In-vitro* drug release of DEX from liposome and solution

## DISCUSSION

When the formulations were evaluated with regard to particles size, polydispersity index and zeta potential, the best formulation was Liposome-A. However, DEX was not sufficiently loaded into Liposome-A. The reason may be that, DEX shows more interest to aqeuous medium by comparison with 0.5 M NaCl containing pH 7.4 phosphate buffer and prefers to stay in outer environment with water instead of loading core of liposome having phosphate buffer.

When we looked at Liposome-B and Liposome-C, we have seen that hydration volume has significant effect on entrapment efficiency of active substances. This is because, as amount of water decreased, entrapment efficiency of active substances increased. Therefore, for the system which had been hydrated with active substance solution after thin film layer formed, using concentrated solution of active ingredient, would be more appropriate. In this way, the liposome will take in large amount of active ingredient with water while liposome is formed.

Different solvan system and different preparation method were both used for Liposome-D, unfortunately IMA did not load into liposome.

As it is shown in Figure 1 and 2, the final release of IMA and DEX from the liposome formulations were found as 21.92% and 23.13% after 72 hours, respectively. This indicates that a slow IMA and DEX release can be observed on similar percentages. Solubility of imatinib mesylate depends on pH and decreases by increasing the pH (Bende, 2008). The reason that the percentage of IMA release did not reach high values at pH 7.4 phopshate buffer may be the pH of receptor medium that is higher than pH of aqueous phase of liposome where IMA is located in liposome and thusly, IMA prefers to stay in liposome core.

As a result of formulation studies, IMA and DEX were succesfully loaded into liposome and obtained liposomes had low particle size distribution and high zeta potential. Franz cell diffusion studies showed us that active subtances were slow relesed from liposomes. Nevertheless, we need further studies that use different system or medium for release to increase percentage of release.

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