*Turk J Pharm Sci 13(1), 69-80, 2016*

**Original article**

# Development of Cisplatin-loaded Liposome and Evaluation of Transport Properties Through Caco-2 Cell Line

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Cisplatin is a potent anticancer drug for treating tumors, that has long been widely used. The therapeutic exploitation of cisplatin is limited by its toxicity toward healthy tissues. Liposomes can provide enhanced efficacy and/or reduced toxicity and these systems offer the potential to enhance the therapeutic index of anticancer agents. In this study, cisplatin liposomes were developed and characterized *in vitro*. The cytotoxicity test was used to determine Caco-2 cell viability and the $IC_{50}$ values of free cisplatin was found 20 µg/mL. Release and transport studies of cisplatin through dialyse membrane and Caco-2 cells were investigated. The stability of liposomes was developed when stored at three different temperature for 3 months. The mean particle size and average zeta-potential of the cisplatin liposomes were approximately 285±0.052 nm and 2.45±0.65 mV, respectively. Cisplatin release from dialyse membrane and transport through Caco-2 cells were obtained as 53.9±2.71 % and 46.2±1.61 % respectively. Significant particle size increase and zeta potantial decrease were not observed in cisplatin liposomes after 3 months when stored at 4ºC ($p>0.01$). Consequently cisplatin liposomes can be delivered orally and represent a potential therapeutic modality in the treatment of tumors.

**Key words:** Liposome, Cisplatin, Caco-2 cell line, Cytotoxicity

## Cisplatin Yüklü Lipozom Geliştirilmesi ve Caco-2 Hücre Hattından Geçiş Özelliklerinin Değerlendirilmesi

Cisplatin yaygın olarak kullanılan etkili bir antikanser ilaçtır. Sağlıklı dokulardaki toksisitesi nedeniyle cisplatinin terapötik kullanımı sınırlıdır. Lipozomlar artan etkililik ve/veya azalan toksisite sağlarlar ve bu sistemler antikanser ilaçların terapötik indekslerini artırmak için potansiyel oluştururlar. Bu çalışmada, cisplatin lipozomları geliştirilmiş ve *in vitro* karakterizasyonu yapılmıştır. Caco-2 hücre canlılığını belirlemek için sitotoksisite testi yapılmıştır ve sisplatinin $IC_{50}$ değeri 20 µg/mL olarak bulunmuştur. Cisplatinin diyaliz membrandan salım ve Caco-2 hücresinden geçişi araştırılmıştır. Üç ay boyunca üç farklı sıcaklıkta saklanan lipozomların stabilitesi değerlendirilmiştir. Sisplatin lipozomlarının ortalama partikül büyüklüğü ve zeta potansiyeli yaklaşık 285±0.052 nm ve 2.45±0.65 mV bulunmuştur. Cisplatinin diyaliz membrandan salımı % 53.9±2.71, Caco-2 hücresinden geçişi % 46.2±1.61 olarak elde edilmiştir. Üç ayın sonunda 4ºC'de saklanan lipozomlarda anlamlı partikül büyüklüğü artışı ve zeta potansiyel azalışı gözlenmemiştir ($p>0.01$). Sonuç olarak cisplatin lipozomları oral yolla kullanılabilir ve tümörlerin tedavisinde olası bir terapötik yaklaşımı temsil etmektedir.

**Anahtar kelimeler:** Lipozom, Cisplatin, Caco-2 hücre hattı, Sitotoksisite

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## INTRODUCTION

Cisplatin is one of the most widely used agents and is a highly effective antineoplastic drug (1) in the treatment of a variety of solid tumors, particularly genitourinary, head and neck, bladder and lung tumors. Cisplatin is a chemotherapy drug utilized clinically and has long been widely used because of its broad spectrum of cytolytic activity against solid tumors (2-6). The use of cisplatin is limited because of its toxicity to normal tissues (5). High dose of cisplatin is difficult to use in practice because of the associated adverse reactions such as renal damage, gastrointestinal disfunction, auditory toxicity, myelosuppression, hematopoietic injury and peripheral nerve toxicity (1,7).

Drug delivery systems can handle these problems, in principle by providing enhanced efficacy and/or reducing toxicity for anticancer agents (8). Cisplatin rapidly passes into the blood circulation and the time period of retention in the tumor is very short. To overcome this problem, drug carrier systems such as liposomes are being investigated because of their favorable characteristics as a biodegradable drug reservoir to prolong retention times (9).

To circumvent cisplatin-related toxicity, drug delivery systems such as microspheres, nanoparticles, unilamellar or multilamellar liposomes are being investigated because of their favorable characteristics (3,9). Drug delivery systems offer the potential to enhance the therapeutic index of anticancer agents, either by increasing the drug concentration in tumor cells and/or by decreasing the exposure in normal host tissues (8). For efficacy and reducing the nephrotoxicity of cisplatin, liposomes are very promising (4).

Liposomes enclose aqueous compartments and are microscopic vesicles composed of one or more lipid bilayers, and can entrap hydrophilic molecules inside or within the lipid bilayers (10-12). Long circulating macromolecular carriers such as liposomes can exploit the 'enhanced permeability and retention' effects for preferential extravasation from tumor vessels (3,8,9). Liposomes can improve safety of drugs, mainly by delivering them to their site of action and by maintaining therapeutic drug levels for prolonged periods. Liposomes can protect bioactive agents from digestion in the stomach and show significant levels of absorption in the gastrointestinal tract (13). For cancer treatment, a number of distinct liposome classes have emerged, based on structural features and associated pharmacologic strategies for delivery. Thus far, the most clinically successful liposomal drugs for cancer treatment have been small unilamellar vesicles (SUV), which consist of a single phospholipid bilayer enclosing an inner aqueous compartment for drug encapsulation. Multilamellar vesicles (MLV) and other structures have been developed as well, and offer an alternative approach to packaging certain drugs (14).

An appropriate *in vitro* cellular model is critical for the better understanding of the cellular and molecular events in response to cisplatin treatment (6). Most studies of drug transport in cell monolayers have been performed using Caco-2 cells (15). The human adenocarcinoma cell line Caco-2 has been developed as a model for the intestinal epithelium. These cells were originally isolated from the human colon adenocarcinoma by Fogh et al. Caco-2 cells are widely accepted and used in in vitro models to predict intestinal absorption by epithelial cells and exhibit enterocyte-like characteristics (10). Caco-2 cells offer a standard rapid, reliable, and low-cost model for in vitro prediction of intestinal drug permeability and absorption (16).

The aim of this study was to develop cisplatin-loaded liposomes and evaluate in vitro characterisation of cisplatin liposome and transport properties through Caco-2 cell line.

## EXPERIMENTAL

*Materials*

Cisplatin (50 mg/100mL) was purchased from Koçak Farma, Dipalmytoilphosphatidilcholine (DPPC) was provided by Across Organics, Belgium. Cholesterol was purchased from Sigma (USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Biochrom, Germany.

*Analytical method and calibration*

UV-spectrophotometric method was used to measure drug content in formulations. Initially absorbance of cisplatin was determined at respective wavelength. Stock solution of cisplatin (100 µg/mL) was diluted from cisplatin market preparation with distilled water. Working standard solutions were prepared by diluting the stock solution in the concentration range from 5-100 µg/mL for calibration curves. Spectrophotometric determination was carried out at 558 nm. The method was found to be linear ($r^2$ = 0.999) and reproducible.

*Preparation of cisplatin liposomes*

Multilamellar cisplatin liposomes were prepared using the dry film hydration method (12). DPPC (20 mg) and Cholesterol (20 mg) were added to the round-bottomed flask in 1:1 molar ratios. They were dissolved with chloroform and evaporated in a rotary evaporator (Heidolph, Germany) under a vacuum at 44ºC until the observation of dry film. The lipid film was kept in desiccators to remove traces of organic solvent. The film was hydrated by a cisplatin commercial solution was diluted with distilled water (100 µg/mL) and liposomes were centrifuged at 15.000 rpm for 30 minutes. Supernatants and liposomes were then separated. Liposome formulations were freshly prepared and used just before the experiments to avoid any degradation.

*Determination of liposome type and characteristics*

The particle size was characterized by dynamic light scattering. Particle size and zeta potential of liposomes were determined using a Zetasizer Nano ZS-(Malvern, UK). Physical appearances of the liposomes were determined using a polarized microscope (Leica DMEP polarized microscope, USA). The cisplatin contents of liposomes were determined by UV spectrophotometer (Shimadzu UV 1700, Japan) from the supernatant phase after ultracentrifugation and separation. Cisplatin amount at the supernatant phase was subtracted from the total.

*Stability Studies*

The stability of suspanded liposome formulations were investigated for 3 months under three different conditions (4ºC, 25ºC+ relative humidity 60 %, and 40ºC+ relative humidity 75 %). In stability studies, we provided these mentioned temperatures with climatic chamber (Sanyo, versatile environmental test chamber, MLR-350H, Japan). Periodically, cisplatin contents, particle size and zeta potential of liposomes were determined every month for 3 months. The test samples were examined during three months and the shelf lives of liposome were calculated for 4ºC, 25ºC and 40ºC, using the Arrhenius equation. The degradation profiles of cisplatin in liposome formulation were examined.

*In vitro release studies of cisplatin from solution and liposomes*

Release studies were performed using Franz diffusion cells with a 12000 Dalton pore size dialysis membrane. A 2.5 mL cisplatin solution and liposome suspension were placed in the donor compartment of the diffusion cells. The receiver compartment was filled with 2.5 mL phosphate buffer (pH 7.4). Cisplatin release studies were performed for 24 h at 37ºC. Samples of 2.5 mL were withdrawn at specific time periods and fresh buffer was immediately replenished at the same volume. The samples were analyzed by UV spectrophotometer as described above.

*Cell Culture Studies*

Caco-2 cell line was obtained from the American Type Culture Collection. The cells were cultivated in a medium composed of DMEM containing 25 mM glucose, 5 mM glutamine supplemented with 10 % fetal bovine serum, 1 % gentamicin, and 7.5 % sodium bicarbonate in an incubator at 37ºC under 5 % $CO_2$ atmosphere.

*Cytotoxicity test*

Cytotoxicity of each test compound was examined. The MTT (3- (4,5-dimethyldiazol-2-yl)-2,5 diphenyl tetrazolium bromide) test assay was used to evaluate the toxic effects on Caco-2 cells.

MTT assay is a colorimetric method for the determination of cell viability based upon

reduction of the yellow tetrazolium salt MTT to a purple formazan dye by mitochondrial succinate dehydrogenase in viable cells (17). The effect of commercial cisplatin solution and formulation components on cell viability was also investigated for a 24-hour time period. The wells containing only the medium were regarded as a positive control with a cell viability of 100%. The color density was measured at 570 nm with a multi-well ELISA reader. The results were calculated as a percentage using the control group values. The drug concentration which inhibited cell growth by 50% ($IC_{50}$) was determined.

*Transport experiments*

Caco-2 cells were seeded at 80,000 cells/$cm^2$ (17) on polycarbonate membranes with a pore size of 0.4 µm (12 mm diameter, 0.4 mm pore size, Costar-Germany). The medium was changed every 48 h for 21 days. The monolayers for the transport studies were used 21 days after seeding on the membranes. The Caco-2 monolayer-containing membrane was placed between the donor and receptor compartments of vertical diffusion cells. Ninety-five percent $O_2$ and 5% $CO_2$ were delivered to the system at 37ºC to maintain cell viability. Transport experiments were performed from the apical to the basolateral compartment. Samples were collected from the basolateral compartment after 15, 30, 45, 60, 90, 120, 150, 240, 360, 480, 720 and 1440 minutes. Cisplatin content of samples was analyzed and apparent permeation ($P_{app}$) values were calculated using following Equation 1 (18,19).

$$P_{app} (\log k) = (dQ/dt) \times (1/AxC_o) \quad \text{Equation 1}$$

dQ/dt refers to the permeability rate, A ($cm^2$) refers to membrane diffusion area, and $C_o$ (mg/mL) refers to the initial concentration of cisplatin in the donor compartment.

*Electrical resistance (cell integrity)*

The integrity of the monolayers was determined by measuring the electrical resistance values (TEER) before and after the experiments. For this purpose, Evohm® Voltmeter was used.

*Statistical Analysis*

All data in this study were considered as means ±SD, and one-way ANOVA was used for statistical analysis. GraphPad InStat ver. 4 was used for the analysis program.

## RESULTS

*In vitro studies*

The type of liposomes were determined using a polarized microscope (Figure 1). Mean particle size, polydispersity index, zeta potentials and encapsulation efficiencies of

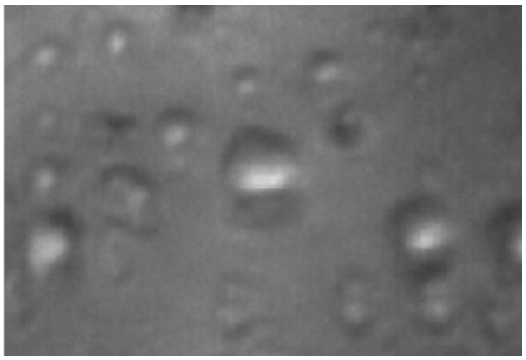


**Figure 1.** Polarize microscope image of liposomes (x40).

cisplatin liposomes were given at Table 1.

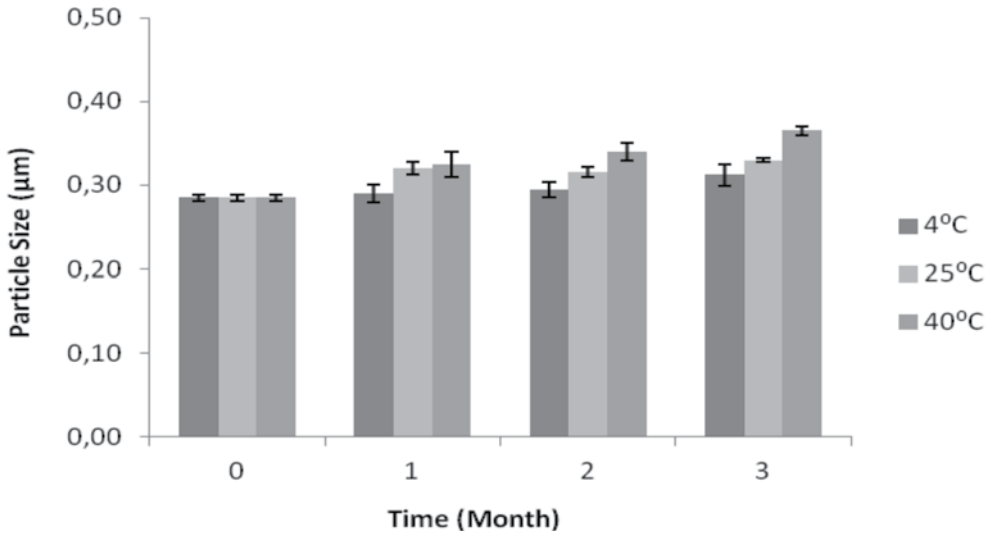
Stability studies were performed at 4ºC, 25ºC+ relative humidity 60 %, and 40ºC + relative humidity 75% for 90 days for liposomes. Mean particle size and zeta potential of the formulations were determined for 3 months. (Figure 2 a and b).

Degradation of cisplatin liposomes followed first order kinetics, and shelf lifes were calculated as 10 days at 4ºC ($r^2$ = 0.954), 6 days at 25ºC ($r^2$ = 0.943) and 4 days at 40ºC ($r^2$ = 0.930) (Figure 3).

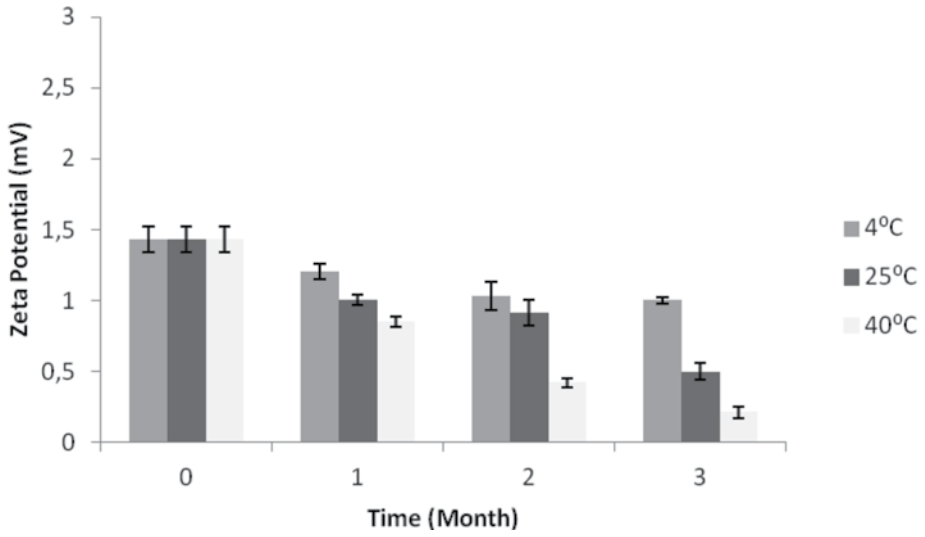
*In vitro* cisplatin release experiment from solution and liposomes formulation were performed with a dialysis membrane using Franz-type diffusion cells and a pH 7.4 phosphate buffer at 37ºC. Cisplatin release from solution and liposomes were found to be with RRSBW (Rosin-Rammler-Sperling-Bennet-Weibull) kinetics, and correlation coefficients were 0.8759 and 0.8890 respectively. Release profiles of cisplatin from liposomes are given on Figure 4.

**Table 1**. Characterisation parameters of cisplatin liposome

| Particle size ±SD (µm) | PDI±SD | Zeta potential ±SD (mV) | Encapsulation efficiency ±SD (%) |
|---|---|---|---|
| 0.285 ± 0.052 | 0.415 ± 0.094 | 2.45 ± 0.65 | 45.1 ± 2.72 |




(a)




(b)

**Figure 2.** The mean particle size (a) and zeta potential (b) of cisplatin liposomes stored at 4ºC, 25ºC and 40ºC (error bars represent standard deviations, n = 3).

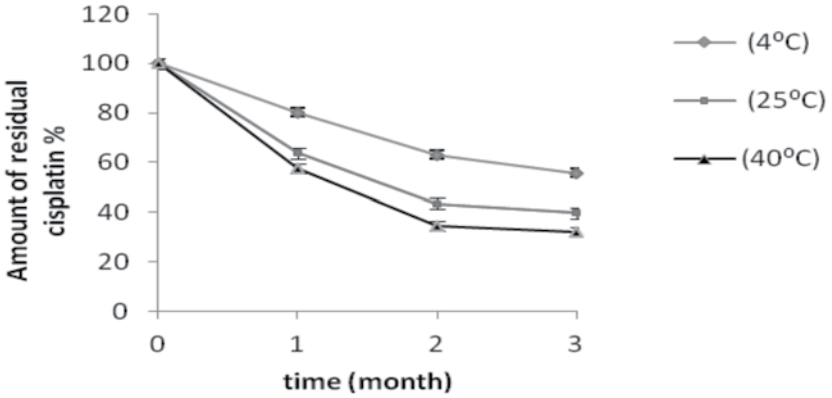



**Figure 3.** Degradation of cisplatin liposomes stored at 4ºC, 25ºC and 40ºC.

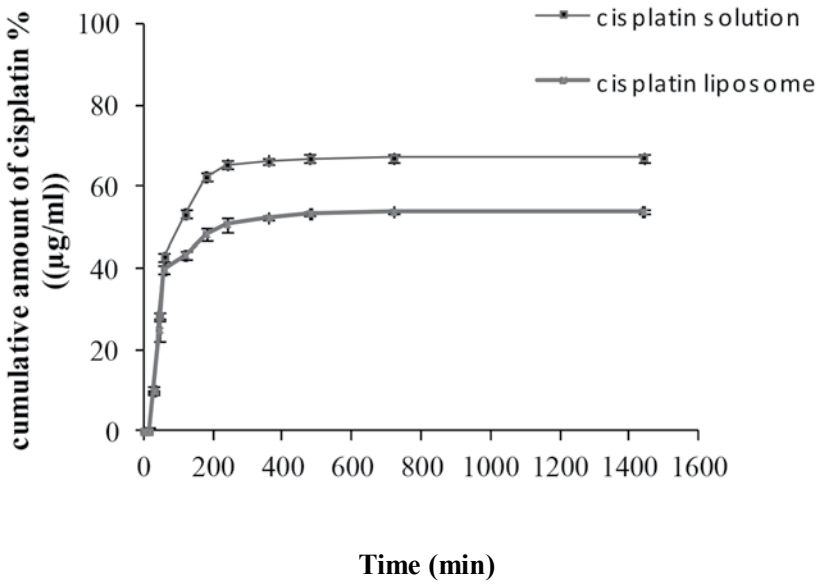



**Figure 4.** *In vitro* release profiles of cisplatin from commercial solution (a) and liposomes (b) at pH 7.4 phosphate buffer (error bars represent standard deviations, n = 3).

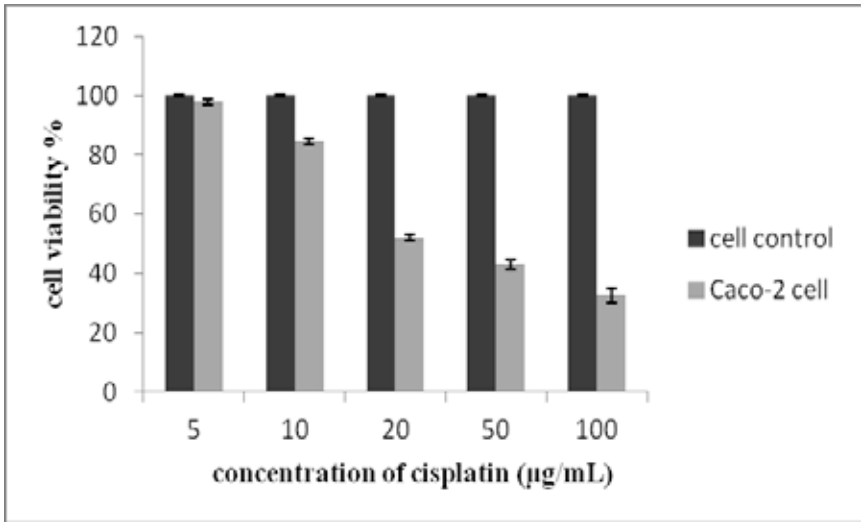
*Cell culture studies*

The MTT assay was used to evaluate the toxic effects on Caco-2 cells. The effect of cisplatin and lipids used in liposomes on Caco-2 cell viability were investigated for 24 h. The wells containing only the medium were regarded as a positive control with a cell viability of 100%. The viability of cells as percentages is given on Figure 5.

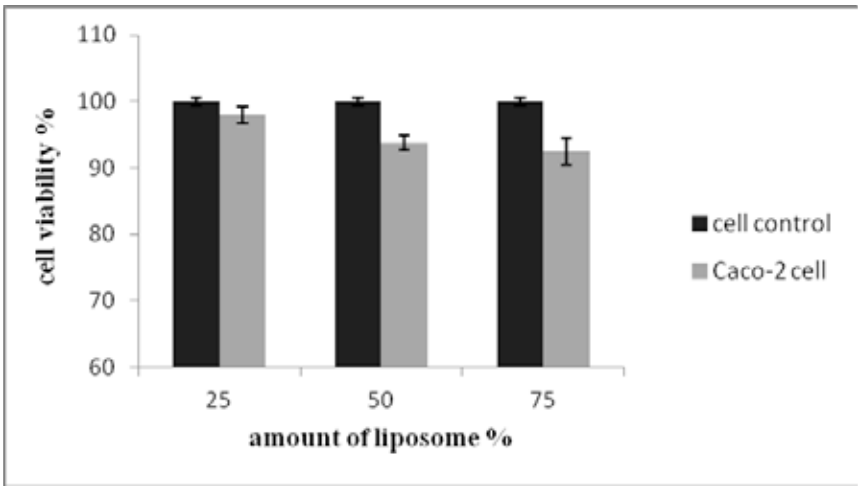
Transport experiments of cisplatin from the solution and liposome through Caco-2 cells from the apical side to the basolateral compartment were evaluated. Cumulative amounts of cisplatin at the end of the 24-hour time period were 58.2 % and 46.2 % respectively. Results are given on Figure 6.

$P_{app}$ (log k) values were calculated for solution and liposome formulations (Table 2).

In clinical use, cisplatin is a chemotherapy drug, that has long been widely used because




(a)




(b)

**Figure 5**. Cisplatin with different concentration on Caco-2 cell viability (a); free liposome with different amount on Caco-2 cell viability (b).

TEER values were found to be 120 ohm before experiment. For cisplatin commercial solution and cisplatin liposome, TEER values were found to be 115 and 113 ohm after experiment respectively.

## DISCUSSION

of its broad spectrum of cytolytic activity against solid tumors. Cisplatin is soluble in water and liposomes enclose aqueous compartments and are composed of one or more lipid bilayers, can entrap hydrophilic molecules inside (12).

In this study, MLV liposomes including cisplatin were prepared by the dry film hydration (20) method using DPPC and cholesterol. The mean particle size and

average zeta potential of the liposomes were approximately 285±0.052 nm and 2.45±0.65 mV, respectively. Liposomal carriers have a strong impact on pharmacokinetics and on the tissue distribution of incorporated drugs. The physicochemical characteristics of the liposomes, such as size, surface charge, steric stabilization affect pharmacokinetic parameters of liposomes. Depending on the size and composition of the liposome, RES uptake can occur within minutes after administration and remove the liposomes around 50% was reported to be quite high for liposomes (22). Therefore, our findings from characterisation appeared to be sufficient.

Liposome formulations have some stability problems, which are lipid oxidation of double bonds, ester bond hydrolysis, and aggregation. For the long-term stability of liposomes, these are the most important disadvantages. The stability of liposomes were studied by examining the particle size, zeta potential and cisplatin amount of liposomes when stored at 4ºC, 25ºC and 40ºC for 3 months. A

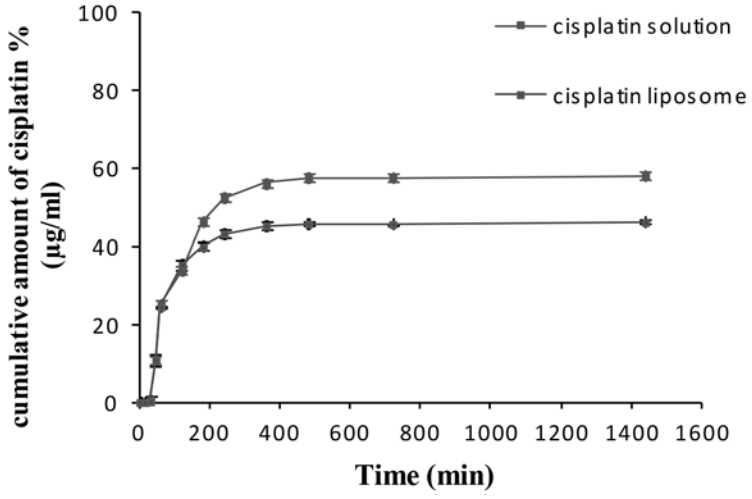



**Figure 6.** Cumulative amount of cisplatin from solution (a) and liposomes (b) transported through Caco-2 cells (error bars represent standard deviations, n = 3).

**Table 2.** Papp (log k) values calculated from Caco-2 transport study results (n = 3)

| Samples | Log k values |
|---|---|
| Cisplatin commercial solution | -1.79±0.070 |
| Cisplatin liposome | -1.60±0.058 |

from the circulation. A liposome with a diameter ~200 nm can remain in circulation for a long time (21). Also to obtain a positive zeta potential is important for interaction with the negatively charged cell membrane.

Encapsulating efficiency of cisplatin liposome was 45±2.72%. It is possible to achieve higher encapsulation efficiency theoretically, but most of the time this cannot be reached practically. The encapsulation efficiency

significant particle size increase was not observed in cisplatin liposomes after 3 months when stored 4ºC ($p>0.01$). The most significant increase at particle size was observed at 40ºC ($p<0.05$). Particles may be subjected to aggregation at 40ºC. Significant particle size increase observed when stored 25 ºC and 40ºC for 3 months ($p<0.02$). The physical stability of liposomes depends considerably on storage conditions. Van der

Waals interactions may cause liposome aggregation at high temperatures (23). Therefore membranes cause the formation of large liposomes by contacting each other. Nevertheless, zeta potential measurement gives information about the storage stability of the colloidal dispersion. Due to the high zeta potential charged particles prevents particle aggregation. Zeta potantial increase observed when stored 25ºC and 40ºC for 3 months significantly ($p<0.05$).

Shelf life of cisplatin liposome was calculated 10 days that was short. In general, freeze-drying increases the shelf-life of liposomal formulations and preserves them in dried form as lyophilized cakes to be reconstituted with water for injection prior to administration (24). Lyophilization may extend the shelf-life of this cisplatin liposomes.

There are many ways to determine cytotoxicity. The MTT test is the most commonly used method (25). The MTT assay indicated that the blank formulation showed no obvious cytotoxicity at various concentrations. According to MTT test results, the $IC_{50}$ values of free cisplatin toward Caco-2 cells was 20 µg/mL. In another study, *in vitro* cytotoxicity of cisplatin was determined on 36 fresh human ovarian cancers. The mean (+standard error) $IC_{50}$ value was 24±5 µg/mL (26). This result is similar to our study data.

Drug release experiments for liposomes are generally performed using a dialysis membrane in buffer solutions. The medium for liposome preparations was selected as pH 7.4 phosphate buffer because the pH of DMEM (cell culture medium) was measured as 7.38 pH. The kinetic releases of cisplatin were found to be with RRSBW for cisplatin commercial solution and liposomes. In this RRSBW kinetic, steeper initial slope followed by a flattened tail in final part was obtained (27).

Caco-2 cells are colorectal derivative epithelial cells. They have been used for studies to test oral absorption and also for the evaluation of different transport mechanisms, such as carrier mediated transport, passive diffusion, and paracellular transport systems (28). Permeability coefficients (Papp) determined for the Caco-2 monolayer have been shown to correlate highly with human absorption *in vivo* (29). Permeability coefficients (log k) were also calculated from solution, and liposome formulation and the lower value was -1.79 cm/h for cisplatin commercial solution, the penetration value found for the cisplatin liposome was -1.60 cm/h.

These results indicate that the membrane structure of liposome, due to the similarities in the cell membrane structures, liposomes are able to penetrate more into the cells and get endositozed easier by the cells. TEER values for solution and liposome were similar before and after experiment. There was no significant decrease in electrical resistance ($p>0.05$).

When the results were evaluated altogether, it can be concluded that liposome containing cisplatin formulation is effective in cancer healing process and it can be an alternative for oral administration. Nanocarrier drug delivery systems have shown their potential to increase the oral delivery of various anticancer drugs. The principal advantages of nanocarriers include their increased solubilization potential, altered absorption pathways, prevention of metabolic degradation within gastrointestinal tract. Also there is no oral administration of cisplatin in clinic, and it can be more effective and can be used for treating tumors.

## CONCLUSION

Delivery systems, as a liposomes may increase their clinical utility for standard anticancer compounds. There is also intense interest in developing delivery strategies for novel anticancer agents that cannot be used by themselves as drugs. The nanocarriers with particle size of 50–300 nm and positive zeta potential were found to have preferential uptake from gastrointestinal tract compared to other nanocarriers.

Liposomes can potentially overcome many common pharmacologic problems such as those involving solubility, stability, pharmacokinetics, tumor uptake, and toxicity. Use of liposomal cisplatin is proceeding in clinical trials since its discovery. Cisplatin liposome formulations, which play more

effective role in cancer treatment, are thought to be promising in cancer therapy.

*In vitro* cellular models are critical for the better understanding of the cellular and molecular events in response to cisplatin treatment. In this study, liposomes were prepared and developed characterisation including cisplatin and investigation transport properties through Caco-2 cell line. Consequently this formulation can be more effective and can be used for treating tumors.

## REFERENCES


1. Song MY, Ku SK, Kim HJ, Han JS, Low molecular weight fucoidan ameliorating the chronic cisplatin-induced delayed gastrointestinal motility in rats, Food Chem Toxicol 50, 4468–4478, 2012.
2. Lv Q, Li LM, Han M, Tang XJ, Yao JN, Ying XY, Li FZ, Gaoa JQ, Characteristics of sequential targeting of brain glioma for transferrin-modified cisplatin liposome, Int J Pharm 44, 1– 9, 2013.
3. Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y, Ultrasound triggered release of cisplatin from liposomes in murine tumors, J Control Rel 137, 63–68, 2009.
4. Guo S, Miao L, Wang Y, Huang L, Unmodified drug used as a material to construct nanoparticles: delivery of cisplatin for enhanced anti-cancer therapy, J Control Rel 174, 137–142, 2014.
5. Mora LO, Antunes LMG, Francescato HDC, Bianchi MLP, The effects of oral glutamine on cisplatin-induced genotoxicity in Wistar rat bone marrow cells, Mut Res 518, 65–70, 2002.
6. Yu HM, Wang TC, Mechanism of cisplatin resistance in human urothelial carcinoma cells, Food Chem Toxicol 50, 1226–1237, 2012.
7. Hirai M, Minematsu H, Hiramatsu Y, Kitagawa H, Otani T, Iwashita S, Kudoh T, Chen L, Li Y, Okada M, Salomon DS, Igarashi K, Chikuma M, Seno M, Novel and simple loading procedure of cisplatin into liposomes and targeting tumor endothelial cells, Int J Pharm 391, 274–283, 2010.
8. Park JW, Liposome-based drug delivery in breast cancer treatment, Breast Cancer Res 4, 95-99, 2002.
9. Hwang TL, Lee WL, Hua SC, Fang JY, Cisplatin encapsulated in phosphatidylethanolamine liposomes enhances the in vitro cytotoxicity and in vivo intratumor drug accumulation against melanomas, J Dermatol Sci 46, 11-20, 2007.
10. Thanki K, Gangwal RP, Sangamwar AT, Jain S, Oral delivery of anticancer drugs: Challenges and opportunities, J Control Rel 170, 15–40, 2013.
11. Hasan M, Belhaj N, Benachour H, Barberi-Heyop M, Kahn CJF, Jabbari E, Linder M, Arab-Tehrany E, Liposome encapsulation of curcumin: Physico-chemical characterizations and effects on MCF7 cancer cell proliferation, Int J Pharm 461, 519– 528, 2014.
12. Yücel Ç, Değim Z, Yılmaz Ş, Nanoparticle and liposome formulations of doxycycline: Transport properties through Caco-2 cell line and effects on matrix metalloproteinase secretion, Biomed Pharmacother 67, 459-467, 2013.
13. Barenholz Y, Liposome application: problems and prospects, Curr Opin. Colloid Interface Sci 6, 66-77, 2001.
14. Mamota C, Drummond DC, Hong K, Kirpotin DB, Park JW, Liposome-based approaches to overcome anticancer drug resistance, Drug Resistance Updates 6, 271–279, 2003.
15. Arturson P, Palm K, Luthman K, Caco-2 monolayers in experimental and theoretical predictions of drug transport, Adv Drug Deliver Rev 64, 280–289, 2012.
16. Qiang Z, Zhong Ye Z, Hauck CA, Murphy PA, McCoy J, Widrlechner MP, Reddy MB, Hendrich S. Permeability of rosmarinic acid in Prunella vulgaris and ursolic acid in Salvia officinalis extracts across Caco-2 cell monolayers, J Ethnopharmacol 137, 1107–1112, 2011.
17. Karamustafa F, Çelebi N, Değim Z, Ünal N, Transport evaluation of alendronate across Caco-2 cell monolayers, Pharmazie 64, 98–103, 2009.
18. Othman AA, Syed SA, Newman AH, Eddington ND, Transport, metabolism and in vivo population pharmacokinetics of the chloro benztropine analogs, a class of compounds extensively evaluated in animal models of drug abuse, J Pharm Exp Ther 320, 344–353, 2007.
19. Ingels F, Deferme S, Destexhe E, Oth M, Van den Mooter G, Augustijns P, Simulated intestinal fluid as transport medium in the Caco-2 cell culture model, Int J Pharm 232, 183–92, 2002.
20. Bangham AD, Standish MM, Watkins JC, Diffusion of univalent ions across the lamellae of swollen phospholipids, J Mol Biol 13, 238–252, 1995.

21. Maruyama K, Intracellular targeting delivery of liposomal drugs to solid tumors based on EPR effects, Adv Drug Del Rev 63, 161-169, 2011.
22. Lasic DD, Novel applications of liposomes, Tibtech 16, 307–321, 1998.
23. Bozkır A, Koçyiğit S, Lipozomların fiziksel ve kimyasal stabilitelerinin incelenmesi, Ankara Ecz Fak Der 24, 1, 1995.
24. Chang HI, Yeh MK, Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy, Int J Nanomedicine 7, 49-60, 2012.
25. Beck RCR, Pohlmann AR, Hoffmeister C, Gallas MR, Collnot E, Svhaefer EF, et al. Dexamethasone-loaded nanoparticle-coated microparticles: correlation between in vitro drug release and drug transport across Caco-2 cell monolayers, Eur J Pharm Biopharm 67, 18–30, 2007.
26. Alberts DS, Fanta PT, Running KL, Adair Jr LP, Garcia DJ, Liu-Stevens R, Salmon SE, In vitro phase II comparison of the cytotoxicity of a novel platinum analog, nedaplatin (254-S), with that of cisplatin and carboplatin against fresh, human ovarian cancers, Cancer Chemother Pharmacol 39, 493–497, 1997.
27. Özkan Y, Savaşer A, Özalp Y, Işımer A, Dissolution properties of different designed and formulated salbutamol tablet dosage forms, Acta Pol Pharm-Drug Res 57(4), 271-276, 2000.
28. Boulenc X, Marti E, Joyeux H, Roques C, Berger Y, Fabre G, Importance of the paracellular pathway for the transport of a new bisphosphonate using the human Caco-2 monolayers model, Biochem Pharmacol 46, 1591–600, 1993.
29. Artursson P and Karlsson J, Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells, Biochem Biophys Res Commun 175, 880–885, 1991.

Received: 23.07.2015
Accepted: 26.11.2015