INFLUENCE OF LIPOSOMAL PLATINUM CYTOSTATICS ON CANCER CELLS BY VOLTAMMETRIC METHODS

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Abstract

Aim of this work deals with closing of cisplatin into liposomes, the study of their stability and interaction with cancer cell lines. Commercial cisplatin cytostatic was encapsulated into three types of liposomes (L8, L10 and L15) of a different chemical composition. Each of liposomes had different ability to close cytostatic. L8 differs from L10 by containing cholesterol. In despite of L10 and L8, L15 liposome contains a synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine ($C_{41}H_{78}NO_8P$) - DOPE, which is generally found in cell membranes. The size of the liposomes was characterized by Dynamic Light Scattering (DLS). The amount of Pt-drug in liposomes was verified by electrochemical determination. Their stability was observed by differential pulse voltammetry (DPV). These studies also demonstrated, that the cisplatin is stable in physiological solution for at least 24 hours, which making it a suitable medium storage for this drug. Finally, the encapsulated cisplatin was applied to cancer cell lines and their viability was observed by MTT assay.

Keywords: Cisplatin, liposomes, electrochemistry, differential pulse voltammetry, cancer cell lines

1. INTRODUCTION

The discovery of anti-carcinogenic drugs containing platinum (Pt), has led in the 1970s to synthesis of compounds containing Pt, which could provide better results in cancer therapy. Unfortunately most of these drugs have toxic side effects; therefore they are given in small doses with low concentration. One of these is cisplatin, which is widely used. However, its antitumor activity is reduced by its side effects. A new form of cisplatin is liposomal cisplatin, which replaces the original one, reducing its toxicity and increasing efficiency. Liposomes are considered to be suitable drug carriers due to their biological inertness, biodegradability, and mainly due to their easy modification of the lipid bilayer surface [1].

2. MATERIALS AND METHODS

2.1. Chemicals

All used chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). High purity deionized water (Milli-Q Millipore 18.2 MΩ/cm, Bedford, MA, USA) was used throughout the study.

2.2. Preparation of liposomes

Lliposome L-8 was prepared by addition of 100 mg of cholesterol ($C_{27}H_{46}O$), 100 mg of 1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) sodium salt ($C_{42}H_{78}O_{10}PNa$) and 100 mg of phosphatidylcholine ($C_{40}H_{80}NO_8P$) into 15 ml tube. Everything was dissolved in 4.5 ml of chloroform ($CHCl_3$). Liposome L-10 was prepared by addition of 100 mg of $C_{42}H_{78}O_{10}PNa$ and 100 mg of $C_{40}H_{80}NO_8P$ into 15 ml tube. Both of them were dissolved in 3 ml

of $CHCl_3$. The preparation of liposome L-15 is the same as preparation of liposome L-8, except that it contains 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine ($C_{41}H_{78}NO_8P$).

Each of these mixtures are pipetted by 150 µl into 20 ml of the tubes and allowed to evaporate. These tubes contain 10 mg of liposome. And then the liposomes were extruded over a filter size about 100 nm. All of three types of liposomes were prepared according to [2].

2.3. Preparation of solutions

Buffer for cisplatin determination was prepared according to method by [3]. For cisplatin solution, were mixed 0.9 g sodium chloride (NaCl) with 100 ml Milli-Q water. Physiological saline solution (0.9 %) with concentration 2.6 g/l was prepared by adition of 26.09 mg cisplatin into 10 ml this solution. Mixture was left 1 hour at 65 °C in 0.9 % physiological saline solution to whole cisplatin dissolution.

2.4. Cisplatin encapsulation into liposomes

For cisplatin encapsulation was used a stock solution with concentration 2.6 g/l according to [3]. From this stock solution, 75 µl (containing 196 µg cisplatin) was collected and mixed with 10 mg of liposomes. Then there was added 225 µl of ethyl ether to open the liposome for cisplatin. The resulting mixture was stirred into a water-oil emulsion in 1:3 ratio. After that, they were evaporated. Besides, 225 µl of physiological saline was added. Finally, this mixture was centrifuged at 4 °C per 1 hour to remove unclosed cisplatin. The supernatant was removed.

2.5. Characterization of cisplatin encapsulation liposomes by DLS

The size of the prepared liposomes was characterized by dynamic light scattering (DLS). The liposomes were extruded through a 100 nm pore filter to achieve the most uniform size. This size is also reported in scientific articles [4, 5]. Prior to measurement, both of the liposomes empty and the encapsulated ones were diluted with 500 µl of MiliQ water due to excessive polydispersity and the ability to detect the size of individual liposomes.

2.6. Electrochemical detection of cisplatin encapsulation liposomes by DPV

Determination of encapsulated liposomes by DPV were performed with 693 VA Computrace instrument (Metrohm, Switzerland), using a standard cell with three electrodes. A hanging mercury (HMDE) drop electrode with a drop area of 0.4 mm^2 was the working electrode. An argentchloride (Ag/AgCl/3M KCl) electrode was the reference and carbon electrode was auxiliary. The analysed samples were deoxygenated prior to measurements by purging with argon (99.999 %). The cisplatin complex was then accumulated on the HMDE surface for 30 s at a potential of −0.5 V with constant agitation. The parameters of the measurement were set as follows: initial potential −0.5 V, end potential −1.2 V, deoxygenating with argon 120 s, modulation time 0.057 s, time interval 0.1 s, step potential 1.95 mV, modulation amplitude 49.5 mV, volume of measurement cell was 2 ml.

2.7. Viability determination of cancer cell lines by MTT assay

This assay is used to determine the viability of cancer cell lines. This method is based on the reduction of the yellow soluble 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) to insoluble formazan. Formazan is subsequently dissolved by the addition of a strong detergent and the purple color is evaluated spectrophotometrically at a wavelength of 570 nm. The absorbance value of the solution is directly proportional to the number of living cells [6]. The result of cell behaviour in individual conditions allows for the generation of a dose-response curve and an IC_{50} determination [7]. The assay was performed on MDA-MB-231 (breast cancer cell line) cell lines, A2780 (ovarian cancer cell line) and UKF-NB-4 (neuroblastoma cancer cell line) and UKF-NB-4-CDDP (neuroblastoma cancer cell line, cisplatin resistant).

3. RESULTS

Electrochemical methods are sensitive to the detection of platinum groups. It was necessary mineralized [8] samples to determine concentration of cisplatin encapsulated liposomes by electrochemistry. We found out, that mineralization mixture during detection decrease peak height of cisplatin. Hence, in this experiment calibration curve was measured in concentration range from 0 µg/l up to 500 µg/l.

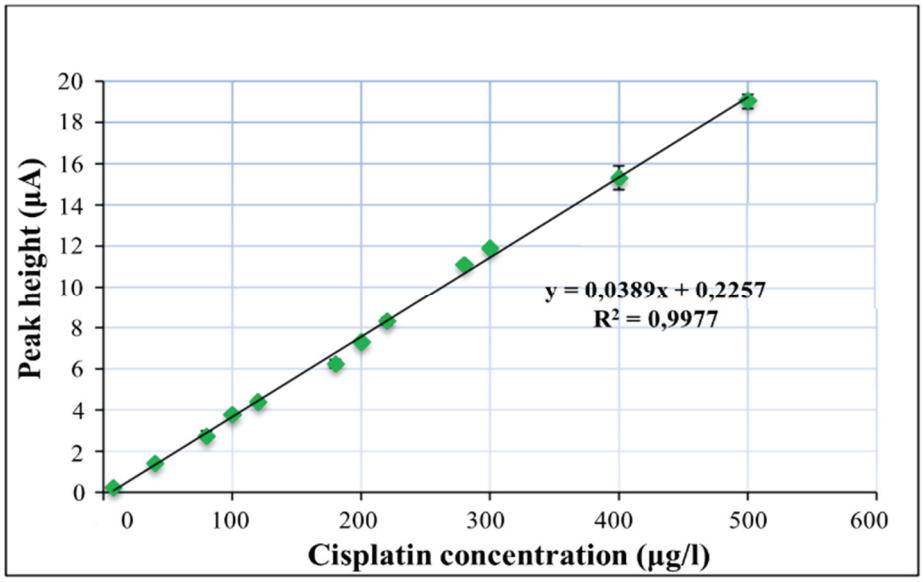

Figure 1 Calibration curve of mineralized cisplatin

Size of extruded liposomes was measured by DLS (NANO-ZS, Malvern Instruments Ltd., Worcestershire, U.K.) during 1 month and also was observed their stability. Size stability of extruded liposomes over the filter is based on study [9]. Liposomes were measured after nine reps.

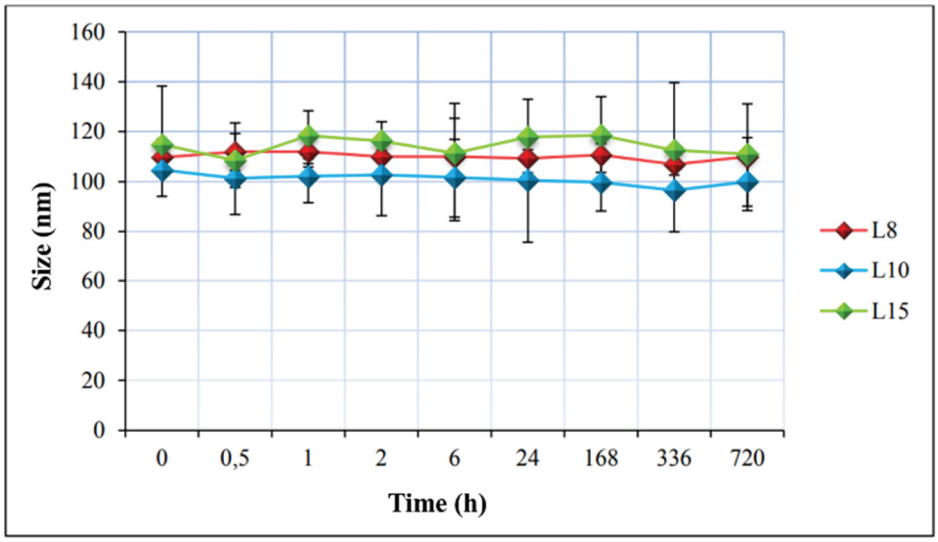

Figure 2 Size change of empty liposomes (100 nm) during month

Moreover, the size of encapsulated liposomes with cisplatin was also measured by DLS, as well as empty ones. Nature of liposomes was studied in different solutions: physiological saline solution, intracellular and in Ringers solution (human plasma analogue).

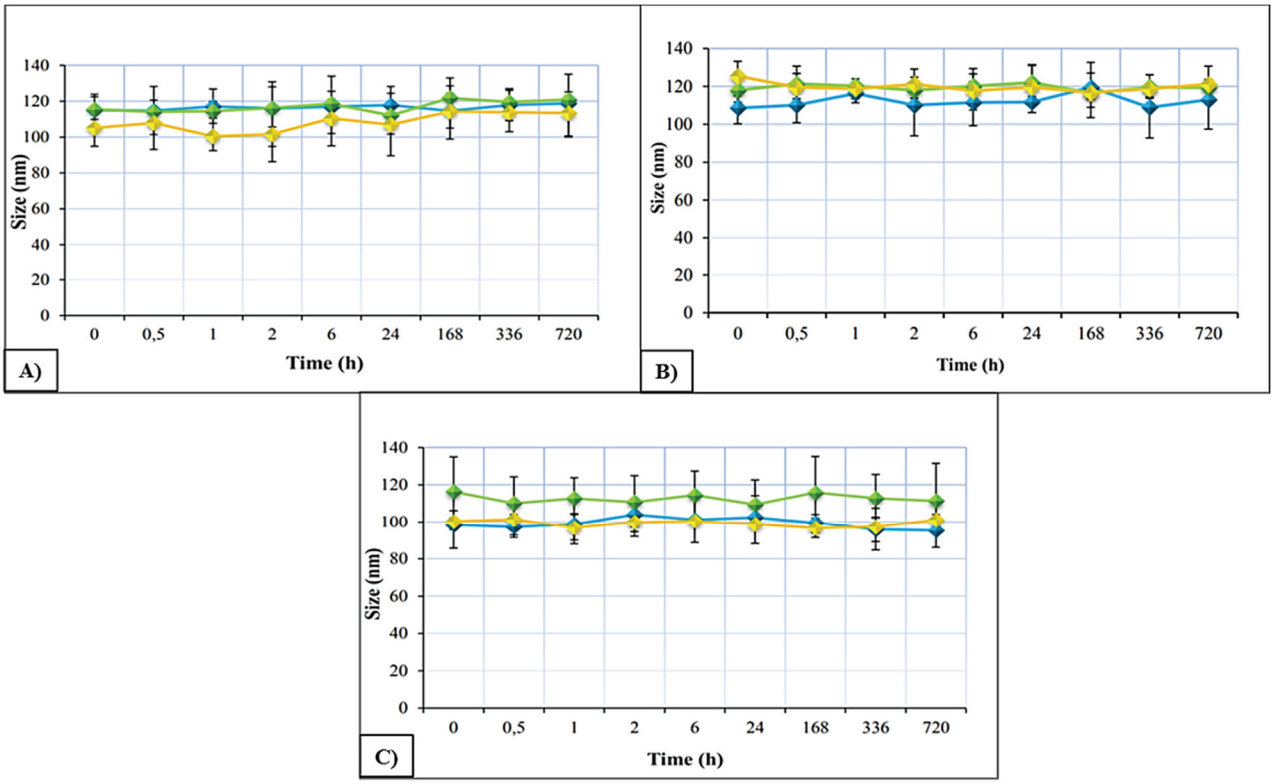

Figure 3 Size change of liposomes with encapsulation cisplatin (100nm) during the month **(A)** liposomes L8, **(B)** L15, **(C)** L10.Blue - physiological saline solution, green - intercellular; yellow - Ringer solution

The device has been set to automatic mode, which means that the device itself selects the optimal position for sample measurement in the cuvette. The size measurements were performed considering a refraction index of 1.456. For size measurement, were used disposable cuvettes type ZEN 0040, containing 50 µL of sample.

The stability of liposomes with encapsulation cisplatin was observed by DPV in 24 hours (h) time framework. Liposomes with encapsulation cisplatin were stable in physiological saline solution. There was a partial cisplatin outflow after six hours, in intracellular. The same effect was observed in Ringer solution.

Table 1 Cisplatin amount (µg) enclosed in liposomes during the 24 hours time Framework in three different solutions

Liposome	Time (h)	Physiological saline solution	Intracellular	Ringer solution
L8	0	12,63	13,68	11,94
	6	17,76	11,69	11,58
	24	17,76	10,88	10,19
L10	0	13,41	14,72	13,59
	6	14,22	13,68	7,19
	24	14,15	8,42	6,28
L15	0	5,45	5,15	4,61
	6	5,81	3,33	15,41
	24	3,58	2,21	15,64

Every dose of cisplatin about concentration 30 µg/ml was applied into cancer cells, because of MTT assay. After that, each of samples were measured by DPV. One control sample (cisplatin itself) was created about concentration 45.2 µg/l. Average amount of cisplatin in encapsulated liposomes: L15 about 7 µg, L8

approximately 14 µg and L10 approximately 11 µg in 10 mg liposome. L15 were unable to enclosed a suffient amount of cisplatin. Therefore, L15 were added in higher amount and it led to increased concentration of cisplatin.

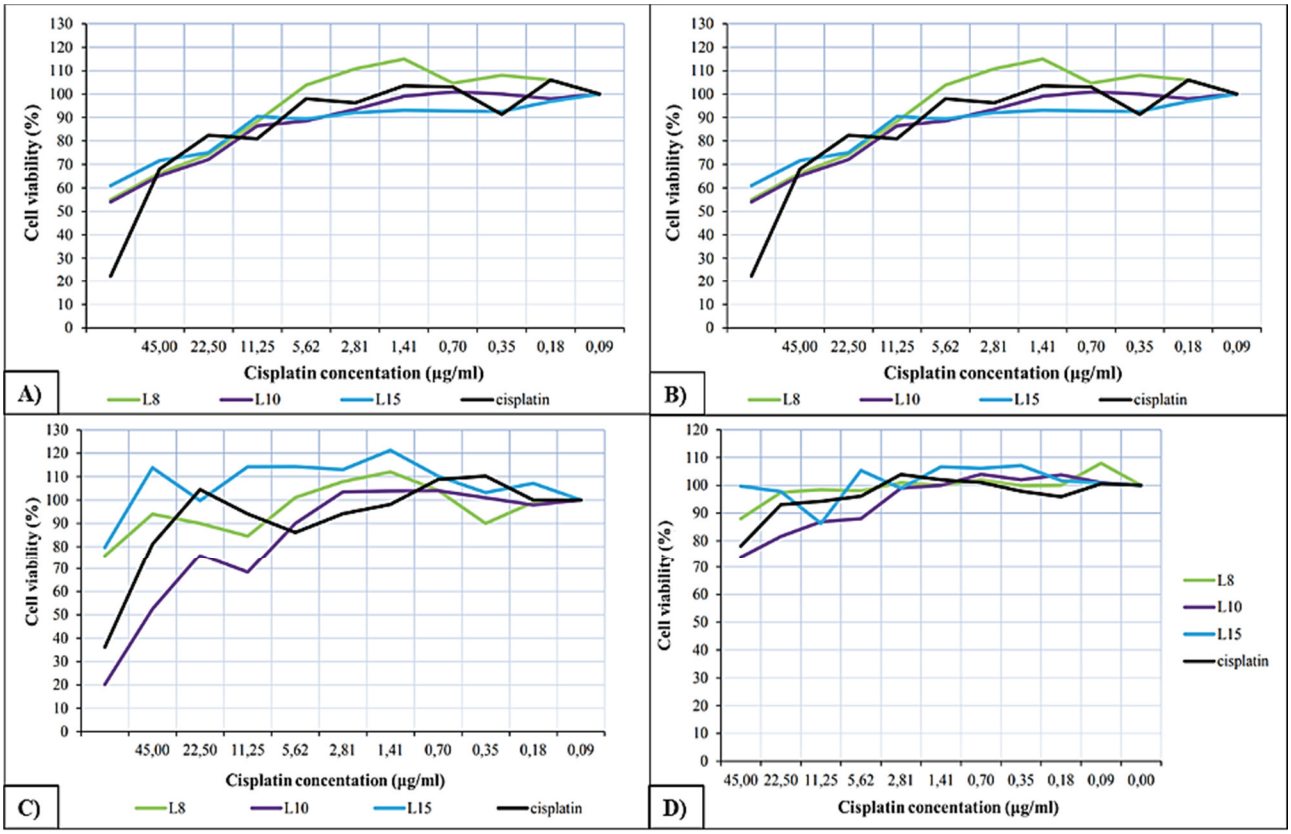

Figure 4 Interaction of cisplatin encapsulated liposomes with cancer cell line **(A)** NB4, **(B)** MDA-MB231, **(C)** A2780 and **(D)** NB4 CDDP

We observed that liposomes L15 had the lowest ability to destroy cancer cell lines. Viability of cancer cells was higher than 50 % in each case. Thus, L15 had shown non-suitable for cisplatin closing. Besides, liposomes L8 weren't supposed to decrease viability of cancer cell lines below 55 %. However they approve good ability to destroy neuroblastoma cell lines. The highest anti-carcinogenic activity was reported by liposomes L10, because of the decreased viability of breast cancer cells under the 45 % and moreover, they decreased viability of ovarian cancer cells below 20 %. However, viability of neuroblastoma cells L10 didn't decreased under the 50 %.

4. CONCLUSION

Aim of this experiment was a voltammetry study dealing with the influence of liposomal platinum cytostatic on cancer cells. We also optimized DPV method for liposomes measurement. The average size of liposomes drug is about 50 - 500 nm [10]. However, too small liposomes can't enclosed enough of cytostatic. In this work, the size 100 nm was chosen as optimal. The size of the prepared liposomes (L8, 10 and 15) was monitored for one month. Their size was evaluated as stable during this time period. All of measurement was done during 24 h. Because it supposed to, that cytostatic interaction with organism, wouldn't be long than 24 h. The highest anti-carcinogenic activity was reported by liposomes L10 applied onto ovarian and brest cell lines.

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