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# Dopamine-loaded liposome and its application in electrochemical DNA biosensor

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**Abstract**

In this study, disruption and lyophilization–rehydration of dopamine-loaded liposome and its application in electrochemical DNA biosensor was investigated. The liposomes containing soyphosphatidylcholine and cholesterol were prepared through thin-layer hydration. First, an investigation was carried out to find an appropriate lysing agent for disruption of prepared liposomes. Differential pulse voltammetry, as a high sensitive electrochemical technique, was used along with a multi-walled carbon nanotubes modified glassy carbon electrode for sensitive electrochemical detection of released dopamine from disrupted liposomes. Various lysing agents were investigated and finally, the disruption of liposomes using methanol was selected without any surfactant, because of its least fouling effect. Then, lyophilization of dopamine-loaded liposomes was carried out using sucrose as cryoprotectant. The electrochemical studies of lyophilized liposomes showed that the remained dopamine in sucrose-protected liposomes was higher than sucrose-free liposomes. Furthermore, sucrose has no interference in electrochemical studies. Then, with the addition of biotin-X-DHPE to liposome formulation, the lyophilized sucrose protected dopamine-loaded biotin-tagged liposomes were prepared and the feasibility of application of them in electrochemical DNA biosensor was investigated as signal enhancer and verified for detection of oligonucleotides.

**Keywords**

Dopamine-loaded liposome, lysing agents, lyophilization, signal enhancer, electrochemical DNA biosensors

## Introduction

Liposomes are nano to micro-sized artificial vesicles composed of lipid bilayers.[1] Their application as drug carriers in pharmaceuticals has been well established[2] and currently, they are constantly used as signal amplification agents in biosensors.[3,4] The capabilities of liposomes as carriers for hydrophobic and hydrophilic compounds resulted in their use as carriers for many signalling molecules such as enzymes, dyes, as well as fluorescent, chemiluminescent, and electrochemical markers.[5] On one hand, the greatest benefits of liposomes in biosensors are obtaining considerable low detection limits and high sensitive assays, and the greatest drawbacks are low stability and leakage of signalling markers due to fusion of liposomes, and on the other hand, low efficiency or less effective methods for disruption of liposomes in bioassays and biosensors.

In assays involving disruption of liposomes, surfactants have been used as cheap lysing agents.[6–12] But the application of them for disruption of liposomes, even in low concentrations cause fouling of electrodes and reduce signal amplification role of liposomes.[7,12] Despite the many studies involving the application of liposomes as signal amplification agents, little attention has been given to investigation on the role of lysing

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agents in assays related to disruption of liposomes and subsequent electrochemical measurements. Furthermore, using of lyophilized liposomes in biosensors just has been suggested in the literature[5,13] but not reported yet. Lyophilization is a promising approach to ensure the long-term stability of liposomes.[14] Moreover, because liposomes are prepared in single step, thus the prepared liposomes have similar characteristics such as loading efficiency and size distribution that can be enhanced the reproducibility of assays. Martorell et al.[15] demonstrated dehydration–rehydration of sulforhodamin B-loaded biotin-tagged liposomes on anti-biotin-coated nitrocellulose strips. The technique was successfully applied to the development of a rapid one-step strip immunoassay for biotin. Furthermore, some electroactive markers including $K_4Fe(CN)_6$, ascorbic acid, and $Ru(NH_3)_6Cl_3$ have been loaded in liposomes and have been used in biosensors.[5]

The preparation and the application of dopamine-loaded liposomes in drug delivery for effective management of Parkinsonism have been reported previously,[16–19] but based on our knowledge, there is not any report on the investigations of disruption and lyophilization of dopamine-loaded liposomes via an electrochemical approach and also there is not any report on the application of lyophilized liposomes in bioassays. Dopamine exhibits a well-known electrochemical behavior, and is a hydrophilic compound[20]; thus, it can be used as an electrochemical marker by loading in liposomes for application in electrochemical biosensors. Moreover, the lyophilization enhances the storage time of liposome dispersions[14]. In this work, differential pulse voltammetry (DPV), as a high sensitive electrochemical technique, along with a multi-walled carbon nanotubes-modified glassy carbon electrode (GCE@MWCNTs) was used for sensitive electrochemical detection of released dopamine from disrupted liposomes. Due to fouling effect of lysing agents on electrode response, first an investigation was carried out to find an appropriate lysing agent, and then the investigation of lyophilization–rehydration of dopamine-loaded liposomes was carried out via voltammetric signal of released dopamine. Finally, the lyophilized–rehydrated liposomes were successfully employed in an electrochemical DNA biosensor for the detection of target oligonucleotide.

## Experimental

### Reagents and materials

Soy phosphatidylcholine (SPC, Lipoid S100, purity = 97.8%) was obtained from Lipoid KG, Ludwigshafen, Germany. *N*-((6-(Biotinoyl) amino) hexanoyl)-1,2-dihexadecanoyl-sn-glycero-phosphoethanolamine, triethylammonium salt (biotin-X DHPE) was purchased from Invitrogen. Dopamine hydrochloride, dimethylformamide (DMF), sucrose, and multi-walled carbon nanotubes (a wall number of 3–15 and a length of 1–10 μm) were purchased from Sigma-Aldrich. Cholesterol, methanol, and chloroform were purchased from Merck. Phosphate buffer saline (PBS) was prepared from proper salts in 1×concentrations (NaCl 137 mM, KCl 2.7 mM, $Na_2HPO_4$ 10 mM, and $KH_2PO_4$ 1.8 mM). Other reagents were obtained commercially and were of analytical grade. Distilled and deionized water was used in all solutions preparations.

All synthetic oligonucleotides were purchased from Bioneer Co. (Seoul, Korea), and were HPLC-purified and freeze-dried products and their sequences are as follows:

Thiolated capture probe: 5′-TCA ACA TCA GTC TGA TAA GCT A-SH-3′
Biotinilated reporter probe: 5′-biotin-Poly $(T)_{20}$-3′
Target: 5′-TAG CTT ATC AGA CTG ATG TTG A-Poly $(A)_{20}$-3′

Capture probe was thiolated from 3′ end which capable its immobilization on $Fe_3O_4$@Au magnetic nano particles (MNPs). Reporter probe is biotin tagged from 5′ end for conjugation to biotinilated liposomes through streptavidin. Target DNA was used as a model oligonucleotide with poly $(A)_{20}$ tail from 3′ end. The required solutions of oligonucleotides (100 μM) were prepared with TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 7.4). The uncapping of disulfide bands of thiol-modified oligos was carried out using a protocol from supplier.

### Instruments

A rotary evaporator (Heidolph, Germany) was used for thin layer preparation. A probe sonicator (Hielscher, USA) was used for ultra-sonication of liposomes. The size and ζ-potential analysis of liposomes diluted by PBS (1×, pH 4.5) was carried out via dynamic light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The morphology of the surface of modified GCE was observed by means of Scanning Electron Microscope (SEM; LEO 440i). A pH meter (827-pH meter, Metrohm, Switzerland) was used for the pH measurements of solutions. Electrochemical experiments were performed using AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie, The Netherlands). A three-electrode system that consisted of glassy carbon electrode (with 2 mm diameter) modified by oxidized multi-walled carbon nanotubes (GCE@MWCNTs) as a working electrode, a saturated

calomel electrode (SCE) as a reference electrode and platinum wire as an auxiliary electrode was used. For dispersing oxidized MWCNTs in DMF, a bath-sonicator was used (sonorex DigiTec, Bandelin, Germany).

### Preparation of working electrode

The multi-walled carbon nanotubes underwent acid treatment by sonication within a 5 h duration in a 1:3 volume ratio of 60% nitric acid and 98% sulfuric acid[21] using a sonication bath. Then, they were washed until the pH of solution reached neutral value. Subsequently, it was dried in air. The surface of glassy carbon electrode (GCE) was cleaned mechanically by polishing with wet 0.05 mm alumina slurry on a felt pad for 1 min. Adherent alumina particles were removed from the electrode surface by rinsing with double distilled water. The GCE@MWCNTs was prepared through dropping of 2 µl of MWCNT dispersion (dispersed in DMF, 6 mg/ml) on the electrode surface and subsequent drying in a chamber at 40°C for 15 min.

### Preparation of dopamine-loaded liposomes

Dopamine-loaded liposomes were prepared through thin-layer hydration.[17] In brief, 10:1 molar ratios of SPC and cholesterol (60 mg total) were dissolved in 8 ml methanol–chloroform mixed solvent (1:3 volume ratio), and then it was transferred to a round-bottom flask. The organic solvents were evaporated using a rotary evaporator under 40°C, 200 bars vacuum, and 150 rpm during 1 h. Liposomes were formed by hydrating the prepared lipid film with 5 ml PBS (1×) pH 4.5, containing 10 mg dopamine hydrochloride. After proper shaking, sonication by a probe sonicator at 60% sonication strength with cycle of 0.5 for two times each for 5 min was used for reducing the size of liposomes. An ice bath was used in sonication step for protecting SPC from overheating. The prepared liposomes were stored at 4°C.

### Lyophilization–rehydration of liposomes

Before lyophilization, liposomes were washed three times to remove unloaded dopamine. For this means, the prepared liposome dispersion was transferred to a dialysis bag immersed in 500 ml of PBS (1×, pH 4.5) and then shacked gently for 15 min in each washing step. For evaluating the washing of liposomes, 100 µl of liposomal solution was transferred to an electrochemical cell containing 2 ml of measurement solution (KCl 0.125 M and phosphate buffer 0.1 M with pH 7.0). Then, the oxidation peak of dopamine was determined using GCE/MWCNTs and DPV technique. The absence of oxidation peak of dopamine was considered as full washing of liposomes (named as *washing test*). Then, two liposomal dispersions were prepared for lyophilization. In one of them, for 5 ml of liposomal dispersion, 1 ml of PBS (1×) containing%10 sucrose was added.[22] For the second liposome dispersion, only 1 ml of PBS (1×) without sucrose was added. Then, the liposomes were transferred to several 2 ml microtubes and kept in a freezer at −60°C. After freezing of liposomes, they were transferred to a vacuum dryer for sublimation of ice. Then, the samples were stored in the freezer at −25°C. For rehydration, the freeze-dried liposomes were reached to initial volumes by adding PBS solution (1×, pH 4.5), and they were sonicated using a bath sonicator for two cycles, each for 5 min.

The lyophilized sucrose-protected dopamine-loaded biotin-tagged liposomes (LSPDLBT-liposomes) were prepared from SPC, cholesterol, and biotin-X-DHPE in 10:1:0.01 molar ratios. The preparation steps are like as biotin-free liposomes but without of washing before lyophilization. The prepared lyophilized liposomes were stored at −25°C. For rehydration, 1 ml of PBS (1×, pH 4.5) was added for each microtube and shacked properly.

### Disruption of dopamine-loaded liposomes

For disruption of liposomes, aqueous and methanolic solutions of Triton-X-100 and Tween-20 (%0.1 and%1) and pure methanol were investigated. In each lysing experiment, 100 µl of liposomal solution was transferred to a micro tube, and then, 200 µl of lysing agent was added to liposome dispersion. Then, the mixture was mixed using a vortex for duration of 30 s. Subsequently, the mixture was transferred to an electrochemical cell containing 2 ml of measurement solution.

### Synthesis of $Fe_3O_4$@Au magnetic nano particles (MNPs) and immobilization of thiolated probe

The $Fe_3O_4$ cores were synthesized through co-precipitation method and Au shell was formed via citrate reduction of Au ions. The details of synthesis of core/shell $Fe_3O_4$@Au magnetic nano particles (MNPs) have been given elsewhere[23] with a little modification. A molar ratio of $Fe_3O_4$ to Au of 2:1 was selected to achieve better Au shell for increasing the possibility of thiolated probe immobilization on them.

The thiolated ss-DNA was uncapped and immobilized on MNPs in which the amount of DNA was 0.11 ng per 1 mg of MNPs. Therefore, the mixture was stirred overnight at 45°C. Then, it was stored at 4°C for further use. The blocking of the surfaces of the

ss-DNA immobilized MNPs were carried out using of 4% BSA solution with 24 h incubation time.

### The description of bioassay

The feasibility of application of LSPDLBT-liposomes in a bioassay was investigated as shown in Scheme 1. At first, in step (A), microtubes were incubated by 2% BSA solution, after washing of them, 50 µl of 0.01 µg/µl streptavidin and 100 µl of 0.4 µM biotinilated reporter probe were added and mixed thoroughly, and incubated for 15 min at room temperature to achieve streptavidin-conjugated biotinilated probe. Then, in step (B), 50 µl of target DNA, and 50 µl of capture probe immobilized MNPs, and 250 µl of hybridization buffer were added to streptavidin-conjugated biotinilated probe, and incubated at 45°C to form MNPs–hybridized DNAs assembly. In step (C), after three times washing of assembly, 200 µl of Tris buffer and then 200 µl of freshly hydrated LSPDLBT-liposome dispersion was added to MNPs–hybridized DNAs assembly and incubated for 15 min to form MNPs–hybridized DNAs–liposomes assembly. Thereafter, the final dispersion of assembly was washed five times to remove any unbound liposomes and unloaded dopamine. Finally, in step (D), in order to disruption of liposomes, 50 µl of deionized water and 100 µl of methanol were added to final precipitate and it was mixed by vortex for 30 s. Subsequently, MNPs were separated by magnet and the solution was transferred to an electrochemical cell containing 2 ml of measurement solution. Thereafter, the DPVs of released dopamine were recorded using MWCNTs/GCE.

### Electrochemical measurements

The DPV measurements were carried out using a positive-going potential scan from 0 to 0.3 V, with a pulse amplitude of 25 mV and a step potential of 5 mV in a 2 ml of measurement solution. Raw data were treated using the Savitzky and Golay filter (Level 2) of the GPES software, followed by the GPES software moving average baseline correction, using a 'peak width' of 0.01. Repetitive measurements were carried out with proper washing and polishing of the GCE surface. The modification of its surface is described in Preparation of working electrode section.

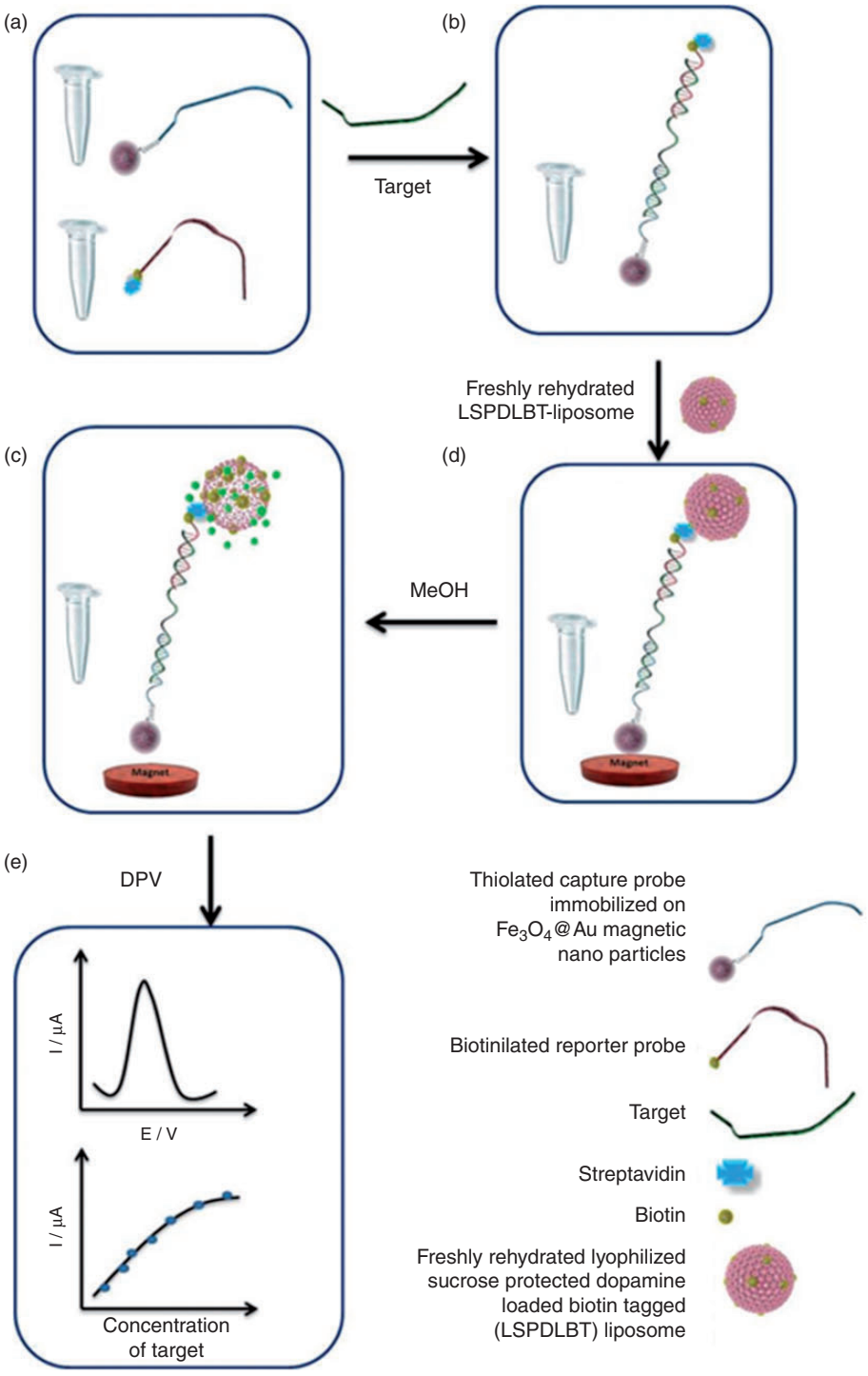
## Results and discussion

### Characterization

*Characterization of liposomes.* DLS is a useful approach for determining the size of liposomes, which gives hydrodynamic diameters of particles dispersed in solution.[24] The ζ-potential is also an important factor that determines the stability of liposomal dispersions. The higher values than 30 mV (both positive and negative values) result in more dispersion stability.[25] For DLS measurements, the values of refractive index and viscosity were considered 1.345 and 1.3 cp, respectively.[26] The results of DLS and ζ-potential for initial and lyophilized-rehydrated liposomes are given in Table 1 (the related graphs have been given in Supplementary data as Figures S1 and S2 for initial liposome and lyophilized liposomes, respectively). As seen the initial liposomes have 266 nm average diameters with low ζ-potential values that lead to the aggregation and sedimentation of them. The variation of the size of liposomes and leakage of the loaded compound are two problems associated with the aggregation of liposomes. These phenomena can reduce the reproducibility of experiments. Thus, it is necessary to prepare liposomes with long-term stability. For this purpose, one way is to increase the electrostatic interactions between liposomes through entering proper compounds in the lipid structure of the membrane,[27] and the other way is utilizing steric hindrance through modification of the surface of liposomes.[28,29] However, both cases may cause difficulties in biosensor applications such as attaching an ss-DNA probe on its surface for subsequent hybridization studies.[30] Moreover, long-term stability of liposomes and also electroactive marker is not attainable even with the mentioned methods due to probable oxidation of dopamine. Thus, lyophilization can be considered as a valuable method in this area. Hence, lyophilization of liposomes was investigated to get a way for long time storage of them. The DLS and ζ-potential analysis of lyophilized–rehydrated liposomes are also given in Table 1. As it was seen, the hydrodynamic diameter for the sucrose-protected sample is 68 nm. For sucrose-free liposomes, two peaks were observed in 58 and 336 nm. Thus, lyophilized-rehydrated liposomes show different size distributions compared with initial liposomes with smaller sizes, due to the secondary sonication step, and a bit agglomeration for sucrose-free liposomes due to the lyophilization step. The ζ-potentials for both lyophilized-rehydrated liposomes are also negative and small values that result in their sedimentation behavior same as initial liposomes.

### Characterization of GCE/MWNCTs surface

Due to unique properties of carbon nanotubes, such as high conductivity, high surface area, and high stability, they have been frequently used for modification of electrode surfaces. Glassy carbon modified by a layer of carbon nanotubes has been frequently used for the sensitive detection of dopamine.[31,32] To verify that dropped




**Scheme 1.** A schematic representation of the application of freshly rehydrated LSPDLBT-liposomes in a bioassay. (a) Blocking of microtubes by BSA, conjugation of streptavidin to biotinilated reporter probe. (b) Formation of MNPs-hybridized DNAs assembly. (c) Addition of LSPDLBT-liposome dispersion to form MNPs-hybridized DNAs-liposomes assembly. (d) The disruption of liposomes with methanol. (e) Electrochemical measurement of released dopamine from conjugated liposomes to assembly.

MWCNTs have been well dispersed on GCE surface; the SEM of modified GCE was taken. As seen in Figure S3, the MWCNTs layer shows well-dispersed porous carbon nanotube layer on electrode surface. This well-dispersed random package of MWCNTs makes not only a high surface area of electrode but also as reported in literature;[33,34] the π–π interactions between MWCNTs and dopamine can enhance its detection.

**Table 1.** Results of DLS and ζ-potential analysis for prepared liposome dispersions.

| Liposome sample | DLS (nm) | ζ-Potential (mv) | |
|---|---|---|---|
| Initial | 266 | −3.32 | |
| Lyophilized–rehydrated | PBS | 58 (%59), 336 (%42) | −0.283 |
| | PBS–%2 ducrose | 68 | −1.21 |

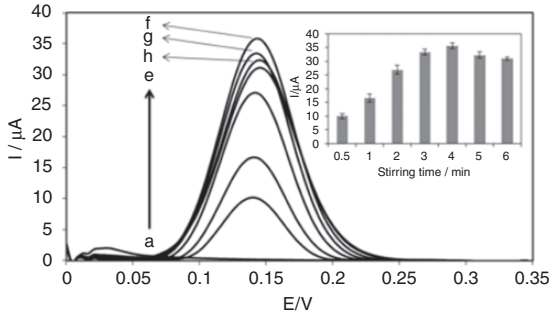



**Figure 1.** Differential pulse voltammograms for different stirring times of (a) blank (b) 0.5, (c) 1, (d) 2, (e) 3, (f) 4, (g) 5, and (h) 6 min for 80 µl of liposome lysed by 160 µl of methanol. Inset: the effect of stirring time on dopamine signal (supporting electrolyte: 2 ml solution containing KCl 0.125 M and phosphate buffer 0.1 M with pH 7.0). Pulse amplitude of 25 mV and a step potential of 5 mV.

## Electrochemical studies

*Developing a reproducible electrode for measuring dopamine.* Since the purpose of this study is to measure the electrochemical signal of released dopamine from disrupted liposomes; thus, for obtaining reproducible results in detection of dopamine, some factors such as the concentration of MWCNTs and its volume, pH of solution, and supporting electrolyte concentration were investigated before. The optimized conditions included concentration of CNT in suspension was 6 mg/ml, volume of dropped CNT suspension on electrode surface was 2 µl, pH of solution was 7.0, and supporting electrolyte concentration was 0.25 M. Reproducibility from electrode to electrode was high with RSD values less than 5%.

## Effect of stirring time on electrode response in measurement of dopamine from disrupted liposomes

Based on the previous reports,[35,36] for a thick-layer modified glassy carbon electrode, both diffusion and adsorption of analyte are accompanied during measurement. Due to the adsorption event, stirring time has significant effects on electrode response. Thus, the effect of stirring time was investigated. For this purpose, the GCE/MWCNTs were immersed in measurement solution. Then, a volume of 80 µl of liposome (lysed by 160 µl of methanol) was added. The solution was stirred in different time cycles and the DPVs of dopamine were recorded. As shown in Figure 1, the stirring time of 4 min, with the highest response was chosen as the optimum value. It should be mentioned that the RSD values for all measurements are less than 5%, unless stated otherwise.

## Choosing an appropriate lysing agent for disruption of liposomes

Due to their low cost, surfactants are the most important agents that have been used for the disruption of liposomes in liposome-based biosensors. Among them Triton-X-100 and Tween-20, both non-anionic surfactants, have been reported.[7–12] However, because of fouling effect, they have a decreasing effect on electrochemical signal. To remedy this problem, one strategy is to develop electrodes resistant to fouling effects of surfactants,[37,38] and the other is to develop better strategies for disruption of liposomes. Thus, here an investigation was carried out on the type and concentration of surfactants in two solvents, aqueous and methanolic, in the disruption of dopamine-loaded liposomes. On one hand, disruption by methanol was also investigated (denoted as methanol only approach). The results are shown in Figure 2. Increasing the concentrations of surfactants in both aqueous (as shown for Tween-20 and Triton-X-100 in Figure 2(a)) and methanolic solutions (as shown for Triton-X-100 in Figure 2(b)) clearly reduces the current. These results show that by increasing the amount of lysing agent, the fouling effect causes reduction in signal. Thus, there is an optimum value for the lysing agent. On the other hand, disruption by methanol without surfactant was also investigated (Figure 2(b), the curve denoted as MeOH). As shown in Figure 2(c), the highest oxidation signal of dopamine was obtained for methanol only approach, and the worst result was for Tween-20 in aqueous solution. Thus, methanol only approach was used for subsequent studies. Since methanol itself has fouling effect, thus, different volume ratios of methanol to liposome were also investigated from 1:1, a slight milky solution, to 3:1, a clear solution after vortexing. The (2:1) ratio of methanol to liposome exhibited higher currents; thus, it was chosen for subsequent studies (the results are not shown here).

## Lyophilization studies of biotin free liposomes

Carbohydrates have been introduced as useful compounds for protecting liposomes during the freezing step.[14] Thus, in this study, sucrose was used as cryoprotectant in lyophilization of dopamine-loaded

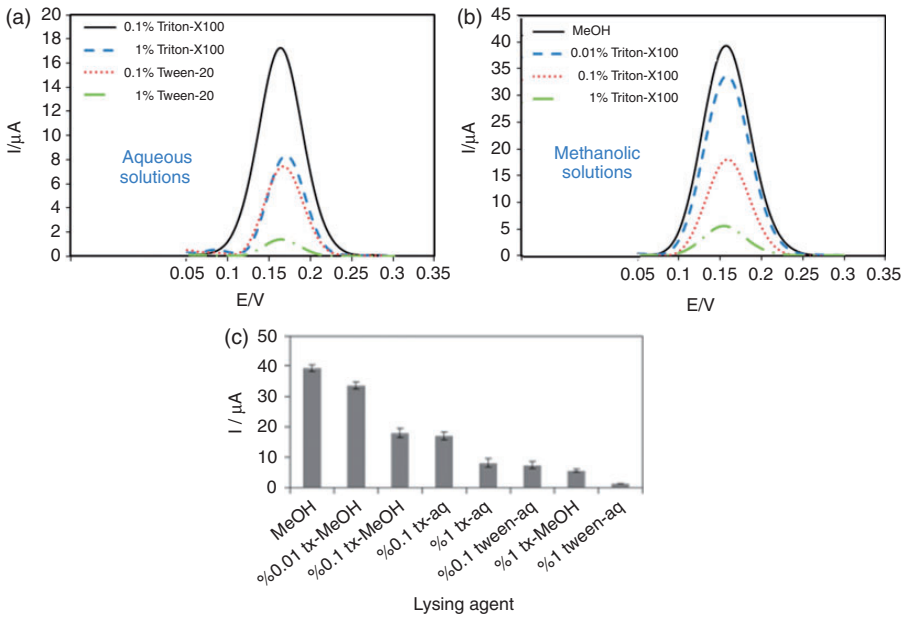



**Figure 2.** Differential pulse voltammograms of dopamine using different lysing agents in (a) aqueous solutions containing various concentrations of lysing agents, and (b) in methanolic solution containing various concentrations of triton-X100 as a lysing agent. (c) A histogram comparison of results of (a) and (b). Experimental conditions as cited in Figure 1.

liposome. Since the addition of sucrose solution or PBS (1×) changes osmolality between the two sides of liposomes membranes, thus it may cause leakage of dopamine. For investigation of this and developing more precise method in lyophilization of dopamine-loaded liposomes, after 30 min of preparation of liposome dispersions, DPVs of dopamine for both liposomal dispersions without lysing were recorded based on the experimental conditions as described in washing test in Lyophilization–rehydration of liposomes section. The results are presented in Figure 3(a). As it was seen, the addition of PBS (1×) did not result in significant leakage of dopamine (curve a), but in the case of sucrose solution, a remarkable peak of dopamine was observed (curve b) that shows the leakage of dopamine due to difference in osmolality between the inner and the outer of liposomes membrane. Thus, in the preparation of lyophilized liposomes, it is necessary to transfer liposomes into the freezer (−60°C) immediately after the addition of sucrose solution to prevent the leakage of loaded dopamine. For confidence that the amounts of loaded dopamine for both liposomes (with sucrose and without sucrose) are the same, the prepared liposomes were disrupted by methanol before lyophilization and the released dopamine was evaluated by DPV. The results are shown in Figure 3(b). As it was seen, the initial amounts of dopamine for both samples (sucrose added and sucrose free) are approximately the same. However, the results of DPVs for lyophilized–rehydrated liposomes without lysing showed a remarkable peak of dopamine (Figure 4(a)) that shows the considerable destruction of liposomes during freeze-drying by crystals of ice.[39]

For evaluating the amount of remained-dopamine in lyophilized–rehydrated liposomes, they were washed with 500 ml of PBS (1×) using a dialysis bag, to remove interference signal from outer dopamine which resulted from the destruction of liposomes during freeze-drying. Subsequently, lysing studies were conducted.

As shown in Figure 4(b), there were DPV peaks for both samples, but a higher signal was observed for the sucrose-protected liposome. Confirming that the amount of dopamine in the sucrose protected liposome dispersion is higher than the other one. Thus, the beneficial role of sucrose as cryoprotectant was verified electrochemically. A comparison between peaks of dopamine, before and after lysing of lyophilized-rehydrated liposomes, shows remarkable leakage of dopamine for sucrose-free liposomes that may be due to the formation a leaky structure of phospholipid bilayer upon rehydration in the absence of cryoprotectant; thus, resulting in the leakage of dopamine during the washing step, too.

Good linear results from standard addition, as shown in Figure 5, confirmed that the presence of sucrose did not interfere with electrochemical studies.

## *Application of LSPDLBT-liposomes in electrochemical DNA biosensor*

After insuring the preparation and electrochemical studies of lyophilized sucrose protected dopamine-loaded

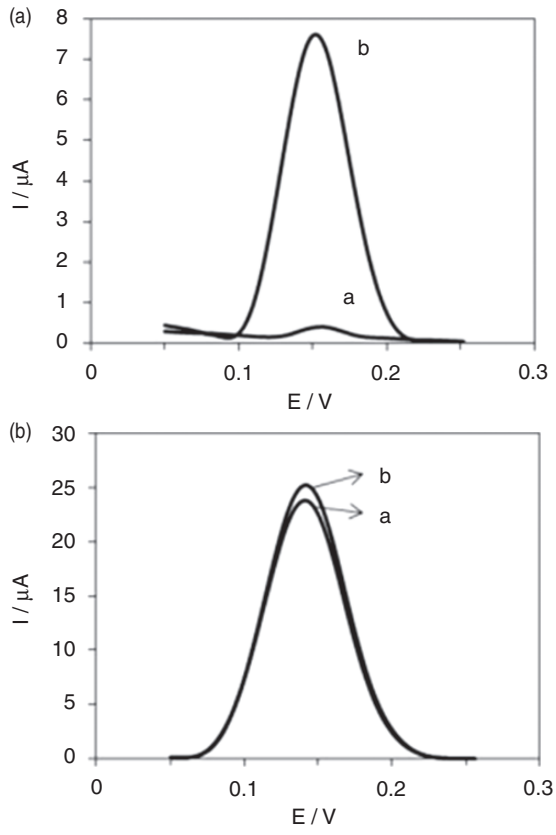



**Figure 3.** Differential pulse voltammograms for prepared liposomes (a) after 30 min, 100 µl of liposome without lysing was added to electrochemical cell, (b) after lysing of prepared liposome but before lyophilization, 50 µl of liposome lysed by 100 µl of methanol and then it was added to electrochemical cell: (a) sucrose-free and (b) in the presence of % 2 sucrose. Experimental conditions as cited in Figure 1.

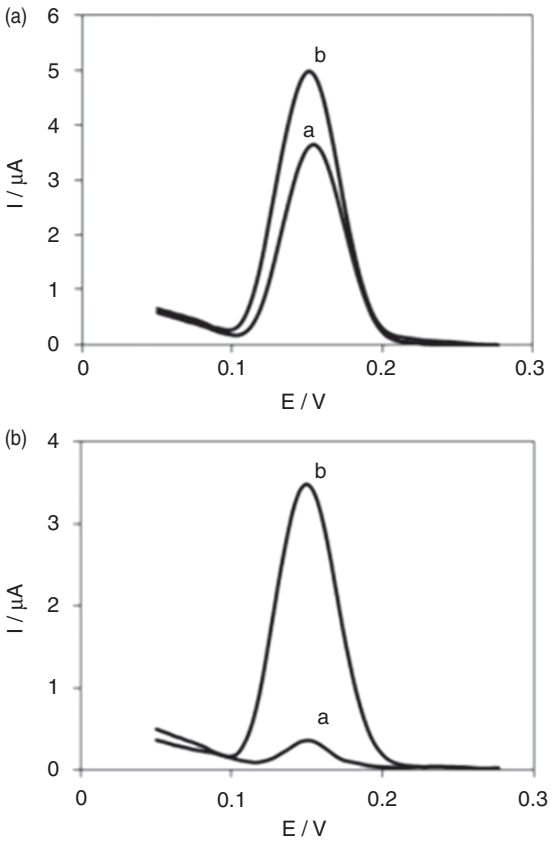



**Figure 4.** Differential pulse voltammograms for lyophilized–rehydrated liposomes (a) without lysing, 10 µl of liposome was added to measurement buffer. (b) After washing and lysing, 50 µl of liposome lysed by 100 µl of methanol and then it was added to measurement buffer: (a) sucrose free and (b) in the presence of % 2 sucrose. Experimental conditions as cited in Figure 1.

liposomes, the LSPDLBT-liposomes were prepared by the same method with adding Biotin-X-DHPE to formulation. The feasibility of application of lyophilized biotin-tagged liposomes in a bioassay was investigated as shown in Scheme 1. The detailed description of bioassay is given in The description of bioassay section. In the investigation of LSPDLBT-liposome performance, the lyophilized liposome were freshly re-hydrated and used. Target ss-DNA with various concentrations was evaluated by described bioassay. With increasing the concentration of target, the peak current of dopamine increased with semi-log behavior (Figure 6). The linear range was 0.01 pM–10 nM with a regression equation of $Ip\ (\mu A) = 1.3579\ \log\ C + 11.442$ ($R^2 = 0.994$), and a detection limit of 5.68 fM ($3S_{y/x}/m$).

These results confirmed the feasibility of application of lyophilized–rehydrated liposomes by using sucrose as cryoprotectant in electrochemical biosensors. Furthermore, these results can be used for developing lateral flow assays with electrochemical detection system.[40] Because of some benefits of the lyophilization

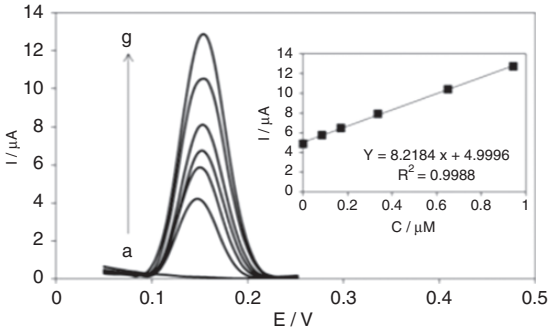



**Figure 5.** Typical differential pulse voltammograms of (a) blank, and the addition of (b) 50 µl liposome disrupted by 100 µl MeOH, and standard addition of (c) 0.086, (d) 0.171, (e) 0.336, (f) 0.650, and (g) 0.945 $\mu$M of dopamine for lyophilized–rehydrated and washed liposome. Inset: related calibration curve. Experimental conditions as cited in Figure 1.

approach, such as long-term stability of liposomes and from the economical viewpoint, it seems lyophilizing is a good approach in liposome-based biosensors that may be useful in future applications.

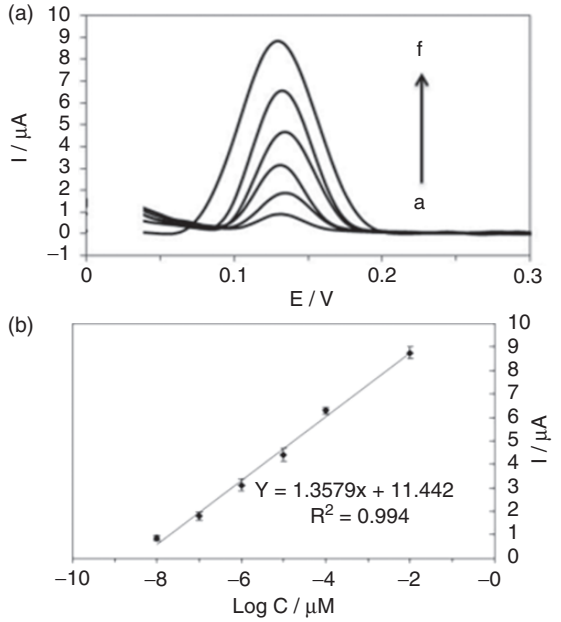



**Figure 6.** (a) Differential pulse voltammograms of (a) 0.01 pM, (b) 0.1 pM, (c) 0.1 pM, (d) 1 pM, (e) 10 pM, and (f) 1 nM of target DNA. (b) Related calibration curve. Experimental conditions as cited in Figure 1.

## Conclusions

In this study, dopamine-loaded liposomes containing SPC and cholesterol were prepared through thin-layer hydration. In order to elucidate disruption and lyophilization of prepared liposomes, first, an investigation was carried out to find an appropriate lysing agent for disruption of liposomes with least fouling effect. DPV, as a high sensitive electrochemical technique, was used along with a GCE@MWCNT for sensitive electrochemical detection of released dopamine from disrupted liposomes. After investigation of some lysing agents, disruption of liposomes using methanol without any surfactant was selected due to its least fouling effect. Then, lyophilization of dopamine-loaded liposomes was carried using sucrose as cryoprotectant. The electrochemical studies of lyophilized liposomes showed that the remained-dopamine in sucrose-protected liposomes was higher than sucrose-free liposomes. Furthermore, sucrose has no interference in electrochemical studies. Then, with the addition of biotin-X-DHPE to liposome formulation, the lyophilized biotin-tagged liposomes were prepared and successfully employed for the detection of oligonucleotides. The proposed strategy in this work could be utilized for detection of real samples. Reports from our laboratory are in progress towards this direction.

### Declaration of Conflicting Interests

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