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Short note

Approach for the electrochemical analysis of hydrophobic compounds included in photo-responsive liposomes

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ABSTRACT

A highly hydrophobic porphyrin: di-octadecyl-amide deuteroporphyrin (D-ODA) was synthesized and incorporated into liposomes (Lipo-D-ODA) through self-assembling of the aliphatic chains. From the fluorescence spectra, it was concluded that the long alkyl carboxylate chains accommodate the sensitizer into the lipid bilayer, is less exposed to quenching induced by aggregation.

The light excited liposome Lipo-D*-ODA activated the ground state molecular oxygen to produce oxygen singlet or superoxide anion.

The electrochemical responses of two structurally different redox-active analytes were studied. Quercetin oxidation at 200 mV was only observed after irradiation on the Lipo-D-ODA/Quercetin, indicating that it is embedded in the liposome and requires membrane rupture. On the contrary, the signal of Ferrocene-ODA was independent of irradiation because the redox polar moieties (Ferrocene) were oriented in aqueous space.

1. Introduction

There has been intense research on lipid vesicles since the 60's due to their capacity for embedding and encapsulating materials, imitating the biological membranes. In this sense, liposomes and micelles of different lipidic composition have been applied in chemical, and biochemical analytics, drug delivery, cosmetics, food technology, and proteomics [1,2].

The liposomes can accommodate hydrophobic molecules inserted into the bilayer by self-assembly during membrane formation and hold entrapped the hydrophilic compounds in the aqueous centre [3]. Most of the studies were carried out in the field of drug delivery, provided that the efficient liberation in a controlled and selective manner is the crucial point of its applicability. The release of molecules embedded in liposomes was triggered by a wide range of physical stimuli, including magnetic or electric fields [4], temperature [5], light [6] and acoustic waves [7]. Electromagnetic irradiation is an attractive stimulus because it is easy to apply and can be localized in time and space. Moreover, the irradiation parameters can be modulated to the system requirements [8].

Efficient solute release from liposomes by electromagnetic irradiation was achieved when they included photoactive molecules, capable of inducing membrane destabilisation and permeabilisation. These physicochemical changes are highly dependent on the intrinsic polarity of the chromophore which affects the localisation in the liposome [9]. One of the most common mechanisms for disruption of membranes is light-induced oxidation by reactive oxygen species (ROS).

Porphyrinic compounds are excellent photosensitizer, in their excited state (Psen*) can interact with molecular oxygen ($^3O_2$) to produce ROS, including singlet oxygen ($^1O_2$), hydroxyl radicals (OH·), and superoxide (O^{2-}) anions which are highly oxidant species capable of deteriorating the phospholipid membrane [10].

The present work reports the preparation and characterisation of a new type of photoactivable liposome, containing a hydrophobic porphyrin. This new material was explored as nanocarrier of electrochemically active molecules of different polarity to be delivered by light stimuli in aqueous solution for their analysis.

2. Experimental details

Soybean phosphatidylcholine (PC) was purchased from Lipoid (Ludwigshafen, Germany). Deuteroporphyrin IX dimethyl ester (D) was from Frontier Scientific. Cholesterol (99%), octadecylamine (97%), quercetin (≥95%), ferrocene carboxaldehyde (98%), 4-aminoantipyrine and phenol were provided by Sigma-Aldrich. 6, 7dioctadecylamide deuteroporphyrin (D-ODA) was obtained by saponification and

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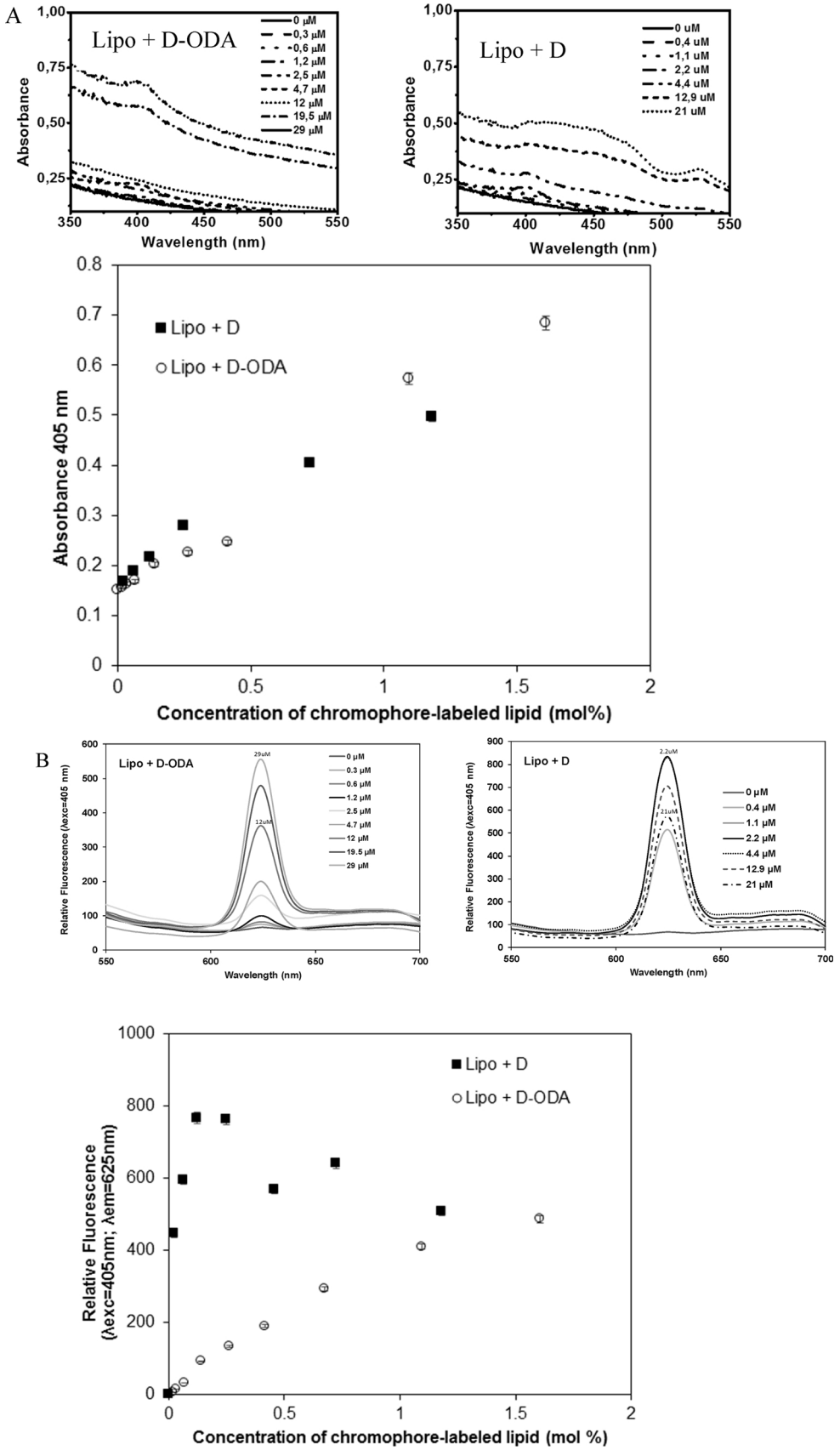

Fig. 1. Changes of (A) absorbance and (B) fluorescence emission spectra as a function of the concentration of deuteroporphyrin (■) and D-ODA (○) in liposomes (mol %).

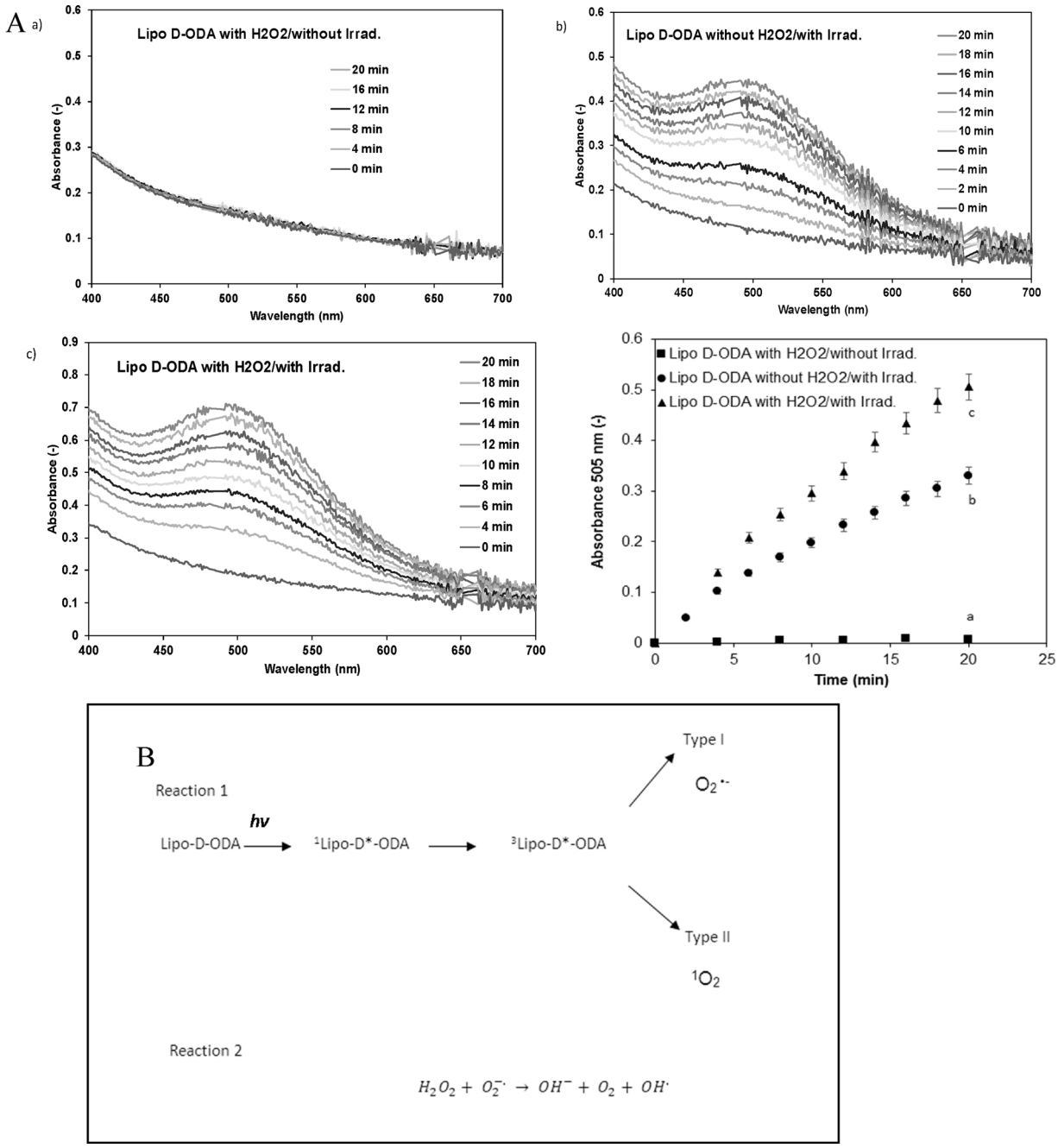

Fig. 2. (A) Absorption spectra and measures at 505 nm of the quinoneimine chromophore versus time, for Lipo D-ODA in the absence (b) or presence (a and c) of 50 µl hydrogen peroxide (50 mM) and with or without irradiation at 405 nm. (B) Reactions involved in the production of ROS.

amide formation reactions (Scheme SI1 in Supplementary material). The MW of the D-ODA is 1013.83011 g/mol (Fig. SI 1). The detailed synthetic procedure of D-ODA is presented in the Supplementary material. Ferrocene octadecylamine (Fc-ODA) was obtained as described in [11].

Intermediate unilamellar liposomes (Lipo) were prepared by the lipid film hydration method [12]. The synthesis of liposomes in the presence of D-ODA, Ferrocene-ODA or quercetin led to Lipo-D-ODA, Lipo-D-ODA/Fc-ODA or Lipo-D-ODA/Quercetin, respectively (Scheme SI2 in Supplementary material).

^{1}H RMN spectra and ^{13}C RMN spectra were determined in $CDCl_3$ and were recorded at room temperature by means of a Bruker AC 600 and Bruker 151 MHz spectrometers. High resolution mass spectra were obtained using electrospray ionization technique and Q-TOF detection (Bruker microTOF-Q II). FTIR spectra were obtained in Thermo Nicolet 600.

The size and shape of liposome loaded with photosensitizers were determined using dynamic light scattering (DLS) and transmission electron microscopy (TEM) (Fig. SI 2) [13].

Spectra absorption of liposomes D-ODA (Lipo-D-ODA) was recorded using HP8452 diode array spectrophotometer. Fluorescence spectrum was obtained using Perkin Elmer LS 45 Fluorescence Spectrometer.

Cyclic voltammetries (CVs) and irradiation with UV light were performed using a potentiostat TEQ4-Z (TEQ-Argentina). A glassy carbon (GC) working electrode (0.25 cm^2 area), an Ag/AgCl reference electrode and platinum wire auxiliary electrode were used for the voltammetric experiment. All CVs measured were carried out directly in 10 mM PBS buffer, 0.1 M NaCl, pH 7.20; a 40 µl drop of sample stock was set onto the GC electrode. The scan rate was 50 mV s^{-1}, in a range voltage between −100 to 700 mV. A 405 nm 50 mW laser line was used

to irradiate samples for cycles of 5 min at 150 mV. Each of one consisted of the use of flashing light 0.5 s on/ 0.5 s off.

The ROS production was explored through the reaction between 4-aminoantipyrine and phenol in the presence of Lipo-D-ODA yielding the corresponding quinoneimine [14]. An aliquot of 2 mL of Lipo-D-ODA solution (dilution ratio 1:4 in phosphate buffer 30 mM pH 7.00) was added to 4 mL of a mixture (v/v) of phenol (0.1 mol/L), 4-aminoantipyrine (1.5×10^{-3} mol/L) and the phosphate buffer at pH 7.0 (0.10 M). The photocatalytic reaction started upon direct irradiation of laser light of 405 nm. The solution was collected and centrifuged, and the supernatant was measured at 505 nm. This procedure was carried out at regular intervals for about 20 min.

3. Result and discussion

A photosensitive material was obtained by the incorporation of a highly hydrophobic porphyrin, D-ODA, into liposomes (Lipo-D-ODA) through self-assembling of the aliphatic chains. Porphyrins are second generation sensitizers [15], producing reactive oxygen species (ROS) by Fenton reaction (H_2O_2 and porphyrins) or by light (porphyrins).

Considering that significant spectral changes are observed upon transfer of a porphyrin molecule from an aqueous phase to the lipid phase, we evaluate the spectroscopic response of D-ODA and deuteroporphyrin IX in the liposome system. Fig. 1A corresponds to the absorbance at 405 nm of the two compounds; the curves are identical, proving that both porphyrins reach the same concentration in the organic medium. On the other hand, the fluorescence spectrum shows a different behavior. Whereas the emission intensity of deuteroporphyrin IX decreases with increasing concentration, due to aggregation-caused quenching (ACQ) [16], the D-ODA response follows a hyperbolic-type curve (Fig. 1B). Clearly, the hydrophobic interactions with the lipidic environment destabilize the aggregated species in favor of the incorporation of the monomer into the liposome, resulting in the significant reduction of the quenching induced by aggregation.

To evaluate the efficiency of production of ROS, we measured the absorption of the chromophore quinoneimine at 505 nm, which is indicative of the efficiency of the reaction of the phenolic substrate and 4-aminoantipyrine. This reaction involves ROS produced in the presence of photoexcited porphyrins. Fig. 2 shows the absorption at 505 nm vs time of irradiation at 405 nm; two different curves are obtained in the absence (b) or presence(c) of 50 µl hydrogen peroxide (50 mM). In both cases, the excited Lipo-D*-ODA could activate the ground state molecular oxygen to produce oxygen singlet or superoxide anion radical according to type II and I mechanism respectively [17]. It is interesting to notice that higher production of ROS is observed in (c), due to the formation of reactive species from H_2O_2 (Fenton reaction). Meanwhile, the curve (a) demonstrates non-production of ROS without irradiation.

To establish a strategy of measurement in an aqueous medium of hydrophobic compounds electrochemically active, we evaluated the analyte release by light excitation of the D-ODA liposome with the light of 405 nm. In Fig. 3, the cyclic voltammetries show different response depending on the structure of the electrochemically active compound, in 10 mM PBS buffer, 0.1 M NaCl, pH 7.20. The quercetin oxidation at 200 mV was only observed after irradiation on the Lipo-D-ODA-Quercetin, indicating that it is embedded in the liposome and requires membrane rupture. On the contrary, Fc-ODA displays an electrochemical signal before the treatment with light, which can be explained considering that the hydrophobic portion (ODA) is embedded in the membrane, and the polar moieties (Fc) oriented to the outer, aqueous space. Furthermore, the ferrocene signal increases after irradiation probably due to the exposition of more redox groups. However, the anodic potential shifts to a more positive value indicating the passivation effect of the aliphatic matrix.

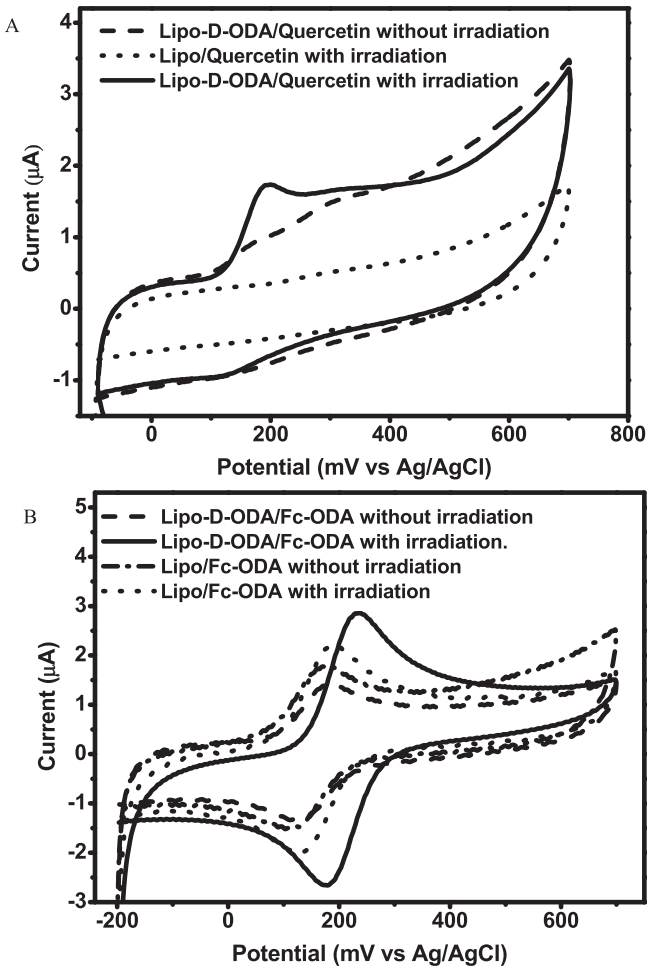

Fig. 3. Cyclic voltammograms obtained during the evaluation of the release of (A) Quercetin (12.6 ppm) and (B) Fc-ODA (21 ppm) in different liposome systems with or without irradiation at 405 nm, in 10 mM PBS buffer, 0.1 M NaCl, pH 7.20. Scan rate 50 mV s^{-1}.

4. Conclusions

A new photosensitive liposome, containing a highly hydrophobic porphyrin as sensitizer was obtained. This new material resulted in an efficient nanocarrier of hydrophobic analytes that were delivered by light stimuli in aqueous solution and further electrochemically analyzed. Considering that biomacromolecules suit well in the lipidic environment, it can be envisioned for future research including membrane proteins, ADN and other macromolecules.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2018.06.010.

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