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Interaction between anaesthetics and model biomembrane systems

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Abstract

Supported layers of vesicles of dimyristoyl and dipalmitoyl phosphatidylcholine (DMPC and DPPC) containing cholesterol (CHOL) are adequate models for eukaryotic plasma membranes. Among the possible substrates to support these layers, gold offers the possibility of being used as an electrode for application in sensors. However, the formation of intact liposome layers on gold is not completely understood and several authors use more or less complex strategies to bind the liposomes.

In this work it was investigated the adsorption of unilamellar vesicles of DMPC, DMPC+CHOL and DMPC+DPPC+CHOL on the surface of oxidized gold using a quartz crystal microbalance with dissipation (QCM-D), atomic force microscopy (AFM) and laser scanning confocal fluorescence microscopy (LSCFM). The results of all techniques indicate that for lipid concentrations ≥ 0,7 mg/ml a dense layer of intact liposomes irreversibly adsorbs on the gold surface.

After the technique of the formation of intact liposome layers was controlled, it was investigated the interaction of some examples of local and general anaesthetics with these membrane models, using a QCM-D. Lidocaine, tetracaine, ropivacaine and levobupivacaine were chosen as local anaesthetics examples, and propofol as general anaesthetic. Simultaneously, the interaction of the anaesthetics with the same liposomes in suspension was studied, using differential scanning calorimetry (DSC) and dynamic light scattering (DLS).

The results showed that all the studied anaesthetics interacted with the used models, causing changes such as raised fluidity, depressed phase transition temperature and increased volume of the lipid bilayer. It was noted that there was a good correlation between the interaction intensity with the lipid membrane and the lipophilicity that is usually used as a measure of the anaesthetic potency.

Keywords: Liposome, quartz crystal microbalance, calorimetry, lidocaine, tetracaine, ropivacaine, levobupivacaine, propofol

Introduction

The first surgical anaesthetics appeared more than 150 years ago, however, their mechanisms of action on cell membranes has not yet been fully elucidated (1; 2). Even today no one knows the main site of action of anaesthetics. There are some authors who claim that the primary site of action are the lipids, others the membrane proteins and even some who believe that they are both (1; 2; 3). The understanding and knowledge of the mechanism of action of any drug is essential to make it safer and more effective, making their study an important challenge.

It is necessary to use good models to study the interaction between anaesthetics and the cell membrane. The supported lipid bilayers are the most widely used models (4), however, the liposomes are a better three-dimensional representation of reality, without ceasing to be a simple but structurally complete model. Knowledge of the mechanisms and critical parameters involved in the formation of supported liposome layers on surfaces has greatly evolved in recent years, however, questions remain regarding the nature of the liposome-surface interactions and the conditions for stability of immobilized liposomes on surfaces (5).

The aim is to contribute to a better understanding of the mechanism of action of anaesthetics and involves two steps:

1. Optimization of the conditions for forming stable liposome layers on gold surfaces;
2. Study the interactions between anaesthetics and liposomes.

In the first stage some factors that may influence the formation of supported liposome layers were studied, including the composition of the liposomes, the optimal concentration to obtain a uniform layer and the influence of temperature. In this step, it was used a quartz crystal microbalance with dissipation monitoring (QCM-D), an atomic force microscope (AFM) and a laser scanning confocal fluorescence microscope (LSCFM). It was also used the technique of dynamic light scattering (DLS) to ensure a uniform liposome size distribution. The QCM-D provides information on the amount of mass adsorbed on the quartz crystal per unit area, as well as some characteristics of the adsorbed film (thickness, elasticity and viscosity). The AFM allows studying the structure of a three-dimensional topographic surface. The LSCFM produces sharp images with high definition, contrast and resolution, of the film adsorbed on the surface of the quartz crystal. DLS determines the size of liposomes.

In the second step we studied the interactions between some examples of local anaesthetics (lidocaine, tetracaine, ropivacaine, levobupivacaine) and a general anaesthetic, propofol, with the liposomes. In this step, we used a QCM-D to investigate the effect of anaesthetics on the immobilized liposomes. The interaction between anaesthetics and liposomes in suspension was assessed with DLS and differential scanning microcalorimetry (DSC). The DSC allows studying the thermotropic behaviour of lipids and the influence of anaesthetics on this behaviour.

Materials and methods

Materials

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol (CHOL) were obtained from Avanti Polar Lipids (Alabaster, AL, USA). N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES), sodium chloride, 5,6-carboxyfluorescein (CF), Triton X-100 solution, chloroform, lidocaine hydrochloride monohydrate, tetracaine hydrochloride, propofol, dimethyl sulfoxide (DMSO) and chloride acid at 37% were from Sigma Chemical Co. Octadecyl rhodamine B (R18) was from Invitrogen. Levobupivacaine hydrochloride and ropivacaine hydrochloride were used as commercial pharmaceutical formulations [Chirocaine from Abbott (pH = 4.47, with sodium chloride, sodium hydroxide, chloride acid and water for sterile preparations) and Naropeine from AstraZeneca (pH = 5.04, with the same excipients of Chirocaine), respectively]. Extran MA 01 (alkaline cleaning solution) was from Merck KGaA and Hellmanex II (alkaline cleaning solution) was from Hellma GmbH & Co. KG. Solutions at 10% and 2% of each detergent were prepared, respectively. All reagents were used as received. The osmolarity of HEPES buffer (10 mM; 0.1 M NaCl; pH = 7.4) was not adjusted because liposome adsorption on gold was found to be practically independent of osmotic stresses (6). Milli-Q water was used in all experiments.

The substrates for immobilization were AT-cut 5 MHz piezoelectric quartz crystals (14 mm in diameter) coated with gold and supplied by KSV Instruments Ltd, Finland and Q-Sense, Sweden.

Methods

Preparation of liposomes

Multilamellar vesicles (MLVs) were prepared from appropriate amounts of DMPC, DPPC and CHOL [DMPC, DMPC+CHOL (70:30 mol%) and DMPC+DPPC+CHOL (35:35:30 mol%)] dissolved in chloroform. After drying the samples under a nitrogen stream, the resulting film was kept under vacuum for at least 3 h to remove all traces of organic solvent. Then, the film was hydrated with HEPES inside a thermostated water bath at ≈ 10 to15 ºC above the temperature of the gel-to-liquid crystalline phase transition (T_m = 24.7 ºC for DMPC and T_m = 41 ºC for DPPC). Heating was alternated with vortex agitation for 1 h.

The obtained MLVs were submitted to 5 freezing-thawing cycles, respectively, in liquid nitrogen and in a water bath at the temperature referred above. Large unilamellar vesicles (LUVs) were obtained from the MLVs by extrusion in a stainless steel homemade extruder, thermostated at the same temperature. The samples were passed several times through

polycarbonate filters (Nucleopore, Whatman) of decreasing pore size (600, 200 and 100 nm; 5, 5 and 10 times, respectively), under inert nitrogen atmosphere. The liposome dispersions were stored at 5 ºC and were used within 2 weeks from preparation.

For fluorescence microscopy assays, DMPC+CHOL vesicles were prepared with R18-labeled lipids and encapsulated CF. Labelling of the lipid bilayer was achieved by addition of 294 μM of R18 to the initial mixture of lipids in chloroform. To encapsulate CF, the lipid film was hydrated in a solution of HEPES containing 50 μM of the dye.

QCM-D

The quartz crystals covered with gold, used as sensor in this work, were washed with a cleaning alkaline solution at 10% (Extran MA 01) in the Ultrasound for 5 minutes, then with Milli-Q water and dried with nitrogen flow. After the drying the crystal was put 10 minutes in UV/Ozone (PSD Serialize UV-Ozone Cleaning & amp; Sterilization of Novoscan), washed again with Milli-Q water and dried with nitrogen flow. Finally, it was put once again 10 minutes in UV/Ozone. The crystals were used immediately after the treatment.

Two QCM-D's were used: one from KSV (model QCM-Z500) and another from Q-Sense (model Q-Sense E4).

In the optimization of the conditions for obtaining a supported layer of liposomes, the experiences were done at 25ºC, 37ºC and 45ºC, in at least three independent measures. In the study of the interaction between the anaesthetics and the liposomes, the experiences were done at 25ºC in at least four independent measurements.

AFM

The topographic characterization was done with a Veeco DI CP-II atomic force microscope. Ultra-sharpened Si tips (MSNL-10 from Veeco Instruments), with a nominal radius of 2 nm and a nominal constant of 0.03 N·m, were used for imaging in Tapping mode. The images were obtained using a liquid micro-cell containing HEPES buffer, at room temperature.

DSC

It was used a differential scanning microcalorimeter (VP-DSC) from MicroCal. The reference and the sample cells have a volume of 0.51 ml. After sample addition, cells were pressurized to 26 psi. The cell temperature was balanced before each scan for 15 minutes. Thermograms were obtained with a heating rate of 60°C/h, between 10ºC and 50°C. At least three cycles of heating/cooling were performed, considering that a cycle consists of a sweep of a heating and cooling scan.

Samples were prepared immediately before doing the tests in order to obtain a suspension of liposomes with a concentration of 0.7 mg/ml. The influence of anaesthetics on the phase transition of model biomembranes was tested with liposome suspension at the concentration mentioned above and the anaesthetic in the concentration studied. Samples were not deaerated to ensure a homogeneous liposome suspension.

The thermograms were subtracted from the baseline corresponding to the buffer and raw data converted into data of molar heat capacity. In the thermograms for pure DMPC liposomes and DMPC liposomes with lidocaine and propofol, a linear function was used to adjust the baseline. In the thermograms of DMPC+CHOL liposomes and DMPC+CHOL liposomes with propofol, a cubic function was used to adjust the baseline. In the other situations the baseline wasn't fitted.

DLS

Size distribution of extruded vesicles was determined at 25 ºC to be 102 ± 5 nm by dynamic light scattering (DLS) using a Spectra Physics model 127 He-Ne laser (632.8 nm, 35mW) and a Brookhaven instrument with a BI-200SM goniometer, a BI-2030AT autocorrelator and a APD detector. Time correlation functions were analyzed by Laplace inversion using the CONTIN method. A lipid concentration of 2 mg/ml was obtained by gravimetry.

Samples were prepared immediately before doing the tests by adding a drop of liposome suspension with a concentration of 0.7 mg/ml to the cell filled with a volume of about 5 ml of HEPES buffer.

LSCFM

Laser scanning confocal fluorescence microscopy images were obtained with a Leica TCS SP5 laser scanning microscope using a HC PL APO CS 0.4 10x dry objective (low magnification) and a HCX PL APO CS 1.20 W 63x water immersion objective (high magnification). The 514 nm line of an Ar laser was used to excite both dyes and the image was simultaneously detected in the regions 530-550 nm (CF) and 580-680 nm (R18). Colocalization software from Leica was used to evaluate the difference in spatial distribution of the two dyes.

Results and Discussion

Preparation of intact liposome layers

QCM-D

The time course of Δf and ΔD upon contact of the gold-coated quartz crystals with the suspensions of DMPC liposomes at several lipid concentrations was recorded at 25ºC. After injection of the liposome suspension, there is a large shift in the resonant frequency accompanied by a large shift in the dissipation which is characteristic of the adsorption of intact liposomes (4; 7; 8; 9; 10). Rinsing with HEPES had a small effect which means that the liposomes are irreversibly adsorbed on the surface. The observed trends of the Δf and ΔD curves are similar to those reported previously for the adsorption of intact vesicles on surfaces of oxidized gold (7) and TiO_2 (11).

For lipid concentrations 0.7 mg/ml, a plateau in the adsorption isotherm seems to be reached. Furthermore, the values of Δf and ΔD stabilize and remain constant during at least 12 hours, suggesting that the immobilized liposomes maintain their three-dimensional configuration. This is the reason why we proceed with the assessment of the influence of the lipid composition by studying adsorbed liposome layers formed from DMPC+CHOL and DMPC+DPPC+CHOL at this concentration.

The influence of the temperature on the DMPC+CHOL liposome adsorption was investigated in the range 25 ºC to 45 ºC, which encompasses the main transition temperature of the mixture, T_m = 27.6ºC. Figure 1 shows Δf/n and ΔD versus temperature obtained after liposomes adsorption onto the gold-coated quartz crystals previously immersed in HEPES, and subsequent rinsing with HEPES, for the third harmonic of the fundamental frequency. The increase in ΔD when the temperature rises may be attributed to a decrease in the vesicles rigidity. This, in turn, affects the contact area between surfaces and vesicles and leads to the release of water trapped between the liposomes with a consequent increase in Δf/n. These results demonstrate the strong tendency of the liposomes to remain intact when adsorbed on the surface of oxidized gold. This behaviour on gold, contrasts with the findings of other authors (6; 9), who claim that the transition temperature of the lipids has a critical effect on the phase transition from surface-bound vesicles to a supported bilayer on SiO_2.

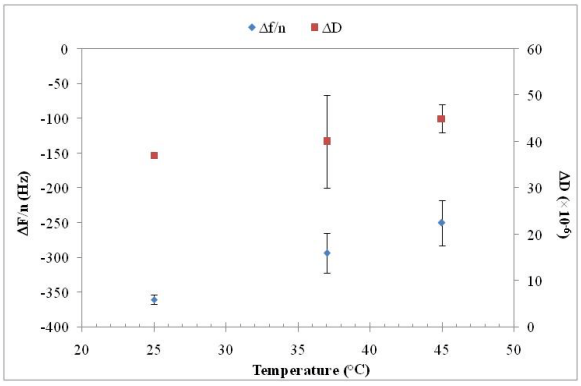


Figure 1 – Temperature dependence of Δf/n and ΔD for the third harmonic of the resonant frequency of a gold-coated quartz crystal after addition of the DMPC+CHOL suspension with lipid concentration of 0.7 mg/ml and subsequent rinsing with HEPES.

AFM

Comparison of the AFM images of the gold surface before (Figure 2a) and after (Figure 2b) immersion in the DMPC+CHOL suspension shows a granular topography in both cases. However, the dimensions of the roundish features are different. On the image obtained after immersion the roundish objects are larger, with average width and height in the order of 120 nm and 10 nm, respectively. The width is slightly larger than the average diameter of the liposomes determined by DLS (102 nm) which may be attributed to the slight deformation of the liposomes caused by adsorption (6). The height of the liposomes is much smaller than the nominal diameter and also smaller than the thickness of the adsorbed layer estimated with QCM-D to be ≈ 68 nm. In fact, according to many reports in the literature (12; 13), the height is usually underestimated because the high

surface density of the liposomes prevents the tip from accessing the substrate. Furthermore, the liposomes appear to be located in different planes which may be caused by rearrangements of the liposomes during imaging, causing a reduction in image resolution.

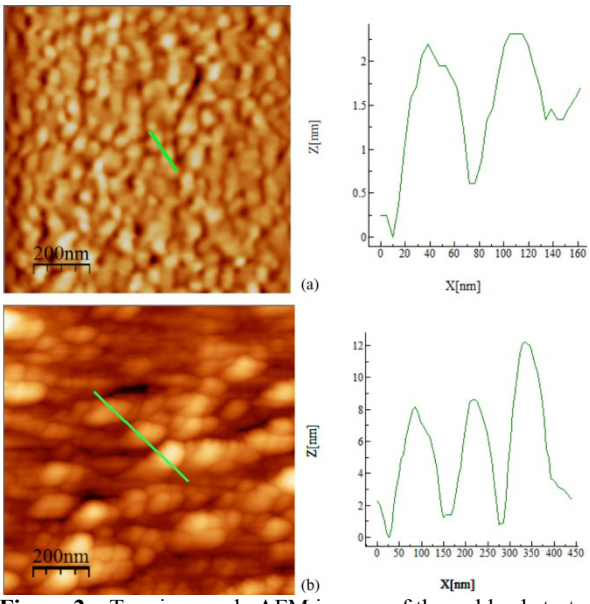


Figure 2 – Tapping-mode AFM images of the gold substrate (a) and of unilamellar DMPC+CHOL liposomes supported on gold (b). Both surfaces have a granular topography but the height profiles show that features on the bare gold surface have average diameters of 60 nm while the liposomes have diameters larger than 100 nm.

LSCFM

The stability of the adsorbed liposomes was further investigated using DMPC+CHOL liposomes prepared with R18-labeled lipids and encapsulated CF. LSCFM images obtained with the liposomes adsorbed on gold-coated quartz crystals are shown in Figures 3a and 3b. The left side of the images corresponds to a region of the quartz substrate with no gold coating, while on the right we can observe the fluorescently labelled liposomes adsorbed onto the gold substrate. In Figure 3a the image was obtained at 530-550 nm, showing the fluorescence emission of CF inside the liposomes, while the image in Figure 3b, collected in the interval 580-680 nm, corresponds to the red-shifted emission of R18 in the lipid bilayer of the liposomes. We observe that some R18 labelled lipids remain adsorbed onto the uncoated quartz substrate (not in the form of liposomes, since no CF fluorescence emission is observed in this region).

In order to better understand the relation between the distributions of R18 labelled lipids and CF labelled vesicles, we show the scatter diagram (Figure 3c) obtained for the two images in Figure 3a,b. The scatter diagram was constructed by assigning a coordinate to every pair of pixels occupying the same position in the two images. Each of these points is marked in the scattering diagram with coordinates equal to the intensity of each point, so that, if a certain pixel has the same intensity in both images, it will lie in the diagonal of the scattering diagram. Each pixel of the scatter diagram has an intensity (red and green scales) showing how often a particular pair of pixels in the images has occurred with that intensity. Completely identical images produce a diagonal line from the bottom left (zero intensity) to the top right (maximum intensity). Differences between the two images cause an irregular distribution in the scatter diagram as observed in figure 3 c,f.

The scatter diagrams in figure 3c shows that the R18 labelled lipids mostly occupy the same region of the image as the CF contained inside the vesicles, as expected for unruptured liposomes. This confirms that the liposomes remain intact after adsorption onto the gold surface, as indicated by the QCM-D and AFM experiments.

However, when an identical sample, with an adsorbed liposome layer, was put into contact with a few drops of a 0.5% Triton X-100 aqueous solution, rupture of the liposomes occurred, with the CF fluorescence disappearing (Figure 3d) after release of the encapsulated CF and dilution in the buffer solution. On the other hand, the fluorescence of R18 was still observed, although a decrease in intensity was observed and the signal disappeared from the uncoated quartz substrate (Figure 3e), suggesting that fragments of the ruptured vesicles stayed bound to the surface but the small amount of lipid previously adsorbed to the quartz (Figure 3b) was effectively displaced by the Triton solution.

The LSCFM results are in agreement with the QCM-D data (not shown) that indicate a significant reduction in the adsorbed mass (increase of $\Delta f/n$ and decrease of ΔD) when the Triton X-100 solution is added to the supported liposome layer.

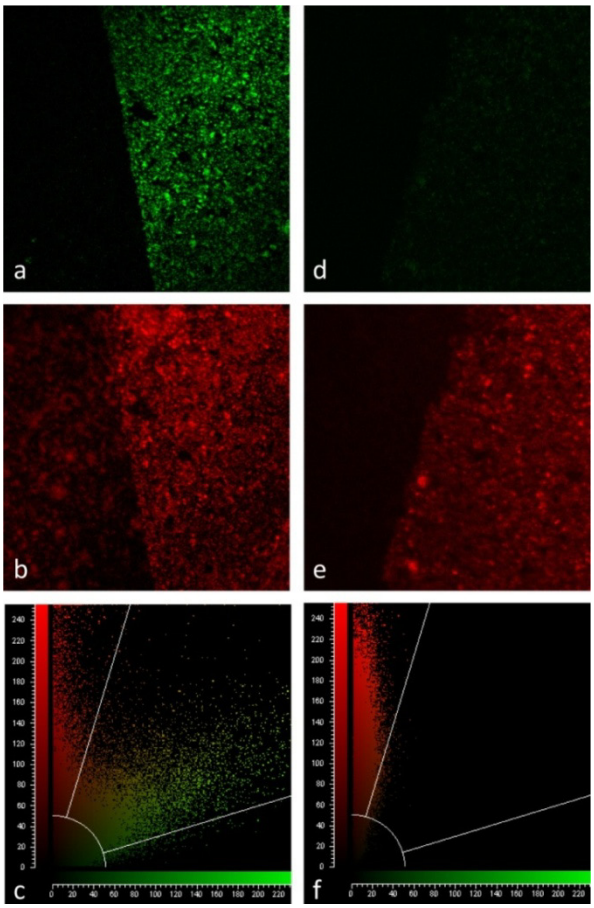


Figure 3 – LSCFM images of R18-labeled DMPC+CHOL liposomes loaded with CF after adsorption on a gold-coated quartz crystal (a, b), and subsequent addition of Triton X-100 (d, e). The scatter colocalization diagram (c) shows that the CF and R18 dyes are mostly localized in the same region of the image. After Triton X-100 solution is added to the system, rupture of the liposomes occurs, with the CF fluorescence disappearing (d).

Study of the interactions between anaesthetics and liposomes

QCM-D

Figure 4 shows the values of Δf/n (figure 4a) and ΔD (figure 4b) for the third harmonic, with respect to the interaction of the studied anaesthetics with DMPC liposomes.

Figure 5 shows the values of Δf/n (figure 5a) and ΔD (figure 5b) for the third harmonic, with respect to the interaction of the studied anaesthetics with DMPC+CHOL liposomes.

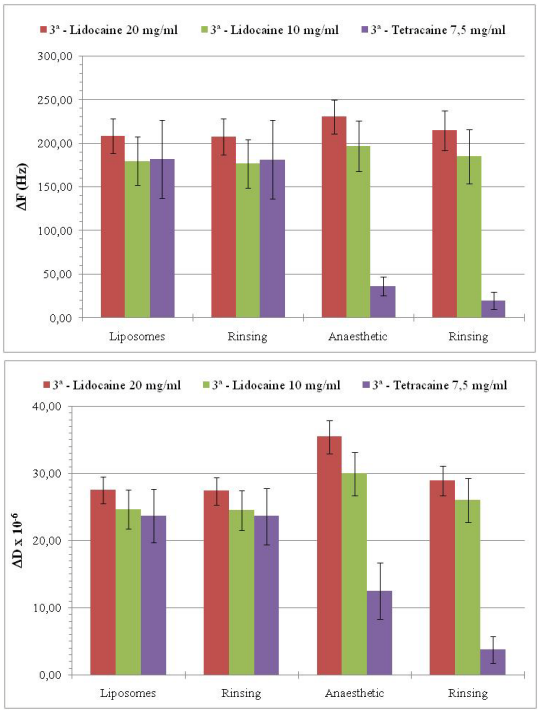


Figure 4 – Change in Δf/n (a) and ΔD (b) for the third harmonic at different stages of the experiment, for DMPC liposomes.

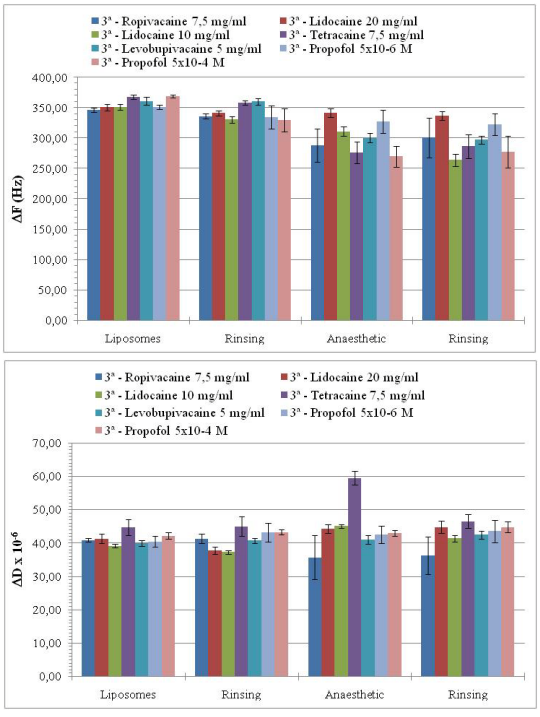
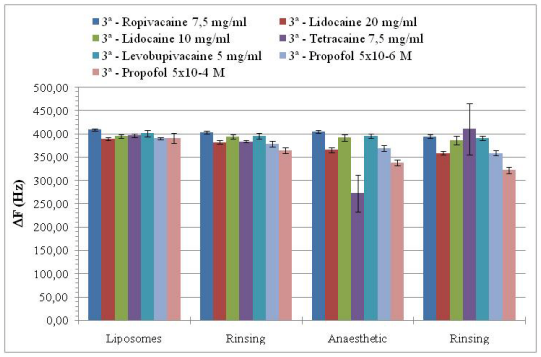


Figure 5 – Change in Δf/n (a) and ΔD (b) for the third harmonic at different stages of the experiment, for DMPC+CHOL liposomes.

Figure 6 shows the values of Δf/n (figure 6a) and ΔD (figure 6b) for the third harmonic, with respect to the interaction of the studied anaesthetics with DMPC+DPPC+CHOL liposomes.

a)



b)

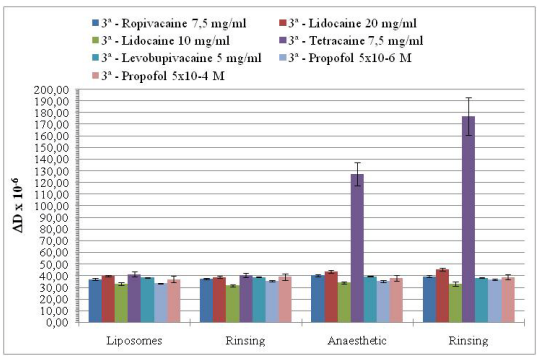


Figure 6 - Change in Δf/n (a) and ΔD (b) for the third harmonic at different stages of the experiment, for DMPC+DPPC+CHOL liposomes.

The consequences of disarrangement of the membrane are, firstly, the possible formation of pores and, secondly, the greater deformability of liposomes. These two phenomena have opposite effects on adsorbed mass change. Water entering the pores causes a mass increase of liposomes immobilized while the deformation of the liposomes and consequent release of water trapped between the adsorbed liposomes leads to a decrease in adsorbed mass. In liposomes without cholesterol, the first effect prevails and the interaction with lidocaine causes an increase in adsorbed mass. In liposomes with cholesterol, which is known to increase the rigidity of the membrane (14; 15; 16), the second effect is much more relevant and therefore the adsorbed mass decreases. The interaction of lidocaine with DMPC+CHOL liposomes and DMPC+DPPC+CHOL liposomes is similar. Another important factor that must be taken into account is the working temperature (25ºC), since at temperatures above the phase transition, the partition coefficient of lidocaine between the lipid and the aqueous phase is higher than below the phase transition temperature (17). For DMPC liposomes, 25ºC is higher than the transition temperaure, while for the liposomes containing cholesterol, 25ºC is lower than the transition temperatures.

The interaction of tetracaine 7.5 mg/ml with supported liposomes confirms the difference in behavior between the liposomes without cholesterol and cholesterol. The interaction of tetracaine with DMPC liposomes causes their disruption and partial removal of the surface. By contrast, cholesterol liposomes seem to resist the interaction with tetracaine although the membrane becomes less rigid which results in loss of solution from the interstices.

Interaction of ropivacaine with liposomes of DMPC+CHOL and DMPC+DPPC+CHOL liposomes is similar but less intense for the latter model membrane. The similarity found between the results of ropivacaine and lidocaine is to some extent expected, given the similarity between the two molecules.

As observed for ropivacaine, the interaction of levobupivacaine with liposomes of DMPC+CHOL and DMPC+DPPC+CHOL is similar but less intense for the latter model membrane. The similarity of behaviour is understandable given the great similarity between the molecules of levobupivacaine and ropivacaine.

The conclusion is that a different behaviour is obtained with the amide-based anaesthetics (lidocaine, ropivacaine and levobupivacaine) and the ester based (tetracaine). Concerning the former ones, the technique of QCM-D does not allow to distinguish between them. However, according to Tomin et al. (18) and other authors (3; 19; 20), the potency of levobupivacaine and ropivacaine is similar but superior to that of lidocaine.

The results obtained with QCM-D at 25 ° C for propofol indicate a decrease of adsorbed mass and an increase in the rigidity of the liposome layer which may be due to the loss of intersticial water due to liposome deformation.

DSC

Figure 7 shows the influence of different concentrations of lidocaine in the thermograms of DMPC liposomes (Figure 7a) and liposomes of DMPC+CHOL (Figure 7b). While the thermograms of the figure 7a have a linear baseline, the thermograms of figure 26b have no baseline.

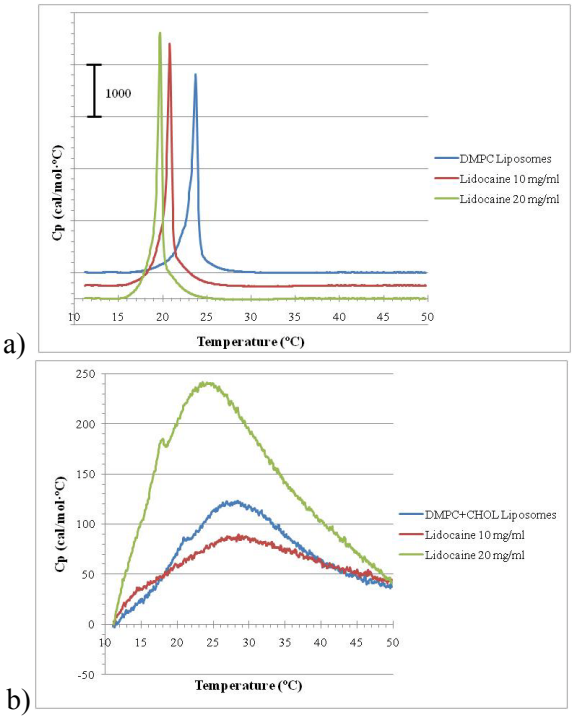


Figure 7 – Influence of different concentrations of lidocaine in the thermograms of DMPC liposomes (a) and DMPC+CHOL liposomes (b).

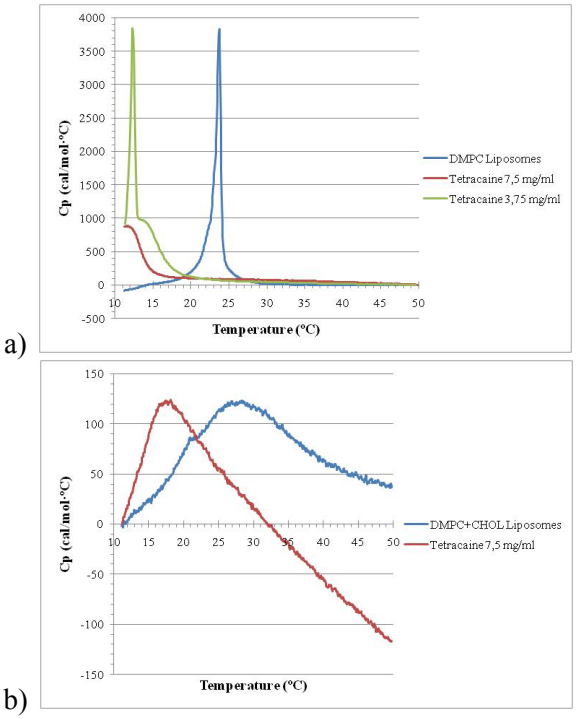


Figure 8 – Influence of different concentrations of tetracaine in the thermograms of DMPC liposomes (a) and DMPC+CHOL liposomes (b).

Figure 8 shows the influence of different concentrations of lidocaine in the thermograms of DMPC liposomes (Figure 8a) and liposomes of DMPC+CHOL (Figure 8b). The thermograms of the figure 8 have no baseline.

Figure 9 shows the influence of different concentrations of lidocaine in the thermograms of DMPC liposomes (Figure 9a) and liposomes of DMPC+CHOL (Figure 9b). The thermograms of the figure 9 have no baseline.

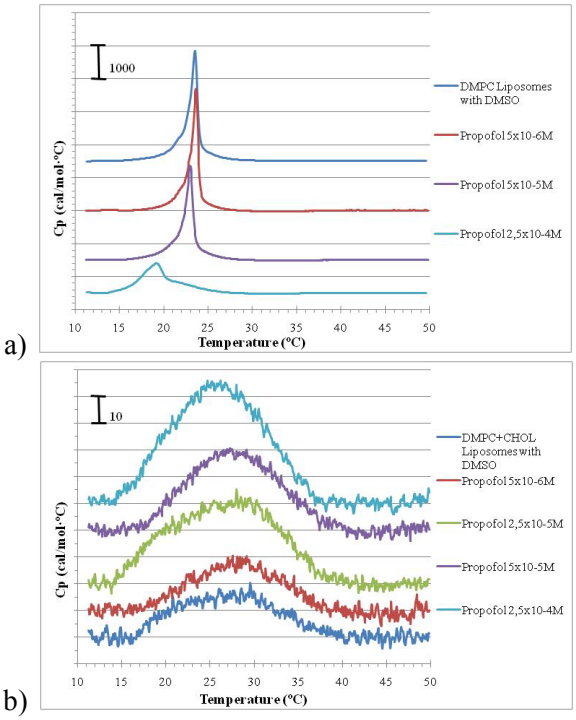


Figure 9 – Influence of different concentrations of propofol in the thermograms of DMPC liposomes (a) and DMPC+CHOL liposomes (b).

After studying the interaction of lidocaine with liposomes of DMPC and DMPC+CHOL it can be concluded, according to the results of calorimetry, that lidocaine penetrates the lipid bilayer and causes a disordering of the hydrocarbon chains (19; 20; 21).

The calorimetry results obtained confirm that a concentration of 7.5 mg/ml causes a huge change in the DMPC membrane that could not be quantified. However, in the presence of cholesterol, it was found that the membrane maintained its integrity even though there was a considerable decrease in transition temperature from gel to liquid state.

Regarding the results obtained with propofol, our data seem to confirm the results of Tsuchiya (21) and Momo et al. (22), who found that propofol causes a decrease in T_m, indicating the fluidizing effect of propofol.

DLS

The size of the liposomes was found to increase with increasing concentration of the anaesthetics, in all cases. The higher increase (265 ± 89 nm) was found for the concentration of 20 mg/ml of lidocaine. These results are in agreement with those of Seeman (23) and Pleuvry (24), who found that anaesthetics increase the volume of the membrane.

Comparison of the various anaesthetics

The results of the studies with the local anaesthetics can be summarized by the following comparison of the intensity of interactions with the three types of liposomes. For the model of DMPC, lidocaine <tetracaine; for DMPC + CHOL, lidocaine <ropivacaine <levobupivacaine <tetracaine, and for DMPC + DPPC + CHOL, levobupivacaine <ropivacaine <lidocaine <tetracaine. This range of interactions can be related to the partition coefficient in n-octanol/water, which allows estimating the potency of anaesthetics. The higher the partition coefficient, the greater the power of the anaesthetic agent. Since the studies of levobupivacaine and ropivacaine were made in an acidic medium, it may be concluded that the potency of these two local anaesthetics may be greater in non-pathological physiological conditions (pH = 7.4). In fact, at a higher pH, the concentration of the ionized form would be lower, thus increasing the neutral species with higher affinity to the lipid bilayer. Thus, the lower the pH the smaller the effect of local anaesthetics (25). For the study of size changes caused by anaesthetics was found that lidocaine induces a greater increase than tetracaine. This difference may be related to the fact that lidocaine cause formation of pores in the lipid membrane that can lead to water ingress into the liposomes. In general, tetracaine is the molecule that interacts more strongly with liposomes while lidocaine is the one with less interaction, leaving the levobupivacaine and ropivacaine in intermediate positions. This sequence is in accordance with their partition coefficients. The only exception is the case of DMPC liposomes DPPC CHOL where interaction with levobupivacaine and ropivacaine is less. As previously stated, at 25°C the ternary mixture is well below the transition temperature from gel to liquid crystalline and therefore should be particularly resistant to the entry of the protonated molecules.

Considering propofol, it is not possible a direct comparison with local anaesthetics, since the concentrations studied were much lower. However, given, for example, the similarity of the QCM-D results obtained with propofol (50 mM) and levobupivacaine (20 mM), it can be concluded that propofol appears to be much more potent. Moreover, the effect of propofol on the transition temperature of gel to liquid crystal is only surpassed by that of tetracaine. Interestingly, the comparison of partition coefficients shows that only the value of tetracaine is greater than that of propofol.

Conclusions

The first objective of this work was to find a simple, reliable method of immobilizing intact liposomes on gold surfaces that could be used as membrane models in future studies of drug-lipid interactions. We chose as model systems unilamellar vesicles with diameter≈ 100 nm of DMPC, DMPC+CHOL and DMPC+DPPC+CHOL. Using QCM-D, tapping-mode AFM imaging and confocal microscopy it was possible to demonstrate unequivocally that, upon immersion inside liposome suspensions in HEPES with lipid concentration of 0.7 mg□mL-1, dense layers of intact liposomes adsorb irreversibly on oxidized gold surfaces. The adsorbed layers remain stable for, at least 12 hours, as long as they are in contact with the liposome suspension or HEPES. The thickness of these layers, which was estimated using the QCM-D software, is compatible with that of a monolayer of deformed liposomes. It was further demonstrated that, even at temperatures above the transition temperature of the mixture DMPC+CHOL, it is possible to adsorb intact liposomes on the gold surface.

The second objective was to study the interaction between some examples of local and general anaesthetics and models of membranes, using the QCM-D technique for liposomes immobilized and DSC and DLS for liposomes in suspension. It was found that all anaesthetics studied interact with the models used, causing changes such as increased fluidity, lowering the temperature of phase transition and increase in volume of the lipid bilayer. The most significant change was obtained with tetracaine, whereas lidocaine was shown to have the slightest effect. It was found that there was good correlation

between the intensity of interaction with the lipid membrane and lipophilicity that usually takes as a measure of anaesthetic potency.

Although this work is still preliminary, it yielded an integrated approach to the study of the interaction of anaesthetics with model biomembranes. Moreover, it provides guiding lines for future investigations, namely the importance of studying the influence of temperature and pH.

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