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Lipophilic prodrug of paclitaxel: Interaction with a dimyristoylphosphatidylcholine monolayer

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ABSTRACT

Interactions between paclitaxel and its squalenoyl prodrug with dimyristoylphosphatidylcholine (DMPC) monolayer at the air/water interface were studied. Paclitaxel is an antineoplastic drug, largely used as anti-cancer agents. Because its low aqueous solubility, Cremophor EL is used as excipient for its formulation. However, it has been shown that Cremophor causes serious side effects. Several attempts have been made to develop a safer formulation such as the synthesis of lipophilic prodrug. In particular we have synthesized a paclitaxel prodrug obtained by conjugation with 1,1,2-trisnorsqualenoic acid to improve the physico-chemical properties of this antineoplastic drug. The miscibility of these compounds with DMPC monolayer were studied analyzing thermodynamic properties as well as excess Gibbs free energies, compressibility modulus and mixed monolayer isotherms. The results allowed to evaluate the spatial organization of the compounds and suggested that the prodrug can efficiently be incorporated in the DMPC monolayer.

1. Introduction

Paclitaxel (taxol) is a natural substance isolated and characterized in 1960s from the bark extract of *Taxus brevifolia* as well as from other species of the *Taxus* genus showing antitumor properties (Fig. 1). The structure of paclitaxel was reported in 1971 but only in 1979 the ability of this compound to promote microtubule assembly in vitro was demonstrated (Wani et al., 1971; Ballatore et al., 2012).

Functionally, paclitaxel binds on α/β-tubulin heterodimers and acts as a microtubule stabilizing agent by inhibiting microtubules dynamic instability and enhancing tubulin polymerization, which promotes mitotic arrest and apoptosis (Schiff et al., 1979). This mechanism of action, toward assembled tubulin heterodimers, was characterized in 1998 by means of electron crystallography (Nogales et al., 1998) and it was shown the binding site of paclitaxel located in the luminal wall of microtubules β subunit, although initial binding of this compound to the outer wall of the microtubule has been proposed (Díaz et al., 2003). In 1992, the U.S. Food and Drug Administration approved paclitaxel for the treatment of ovarian cancer (Eisenhauer and Vermorken, 1998) and two years later it was approved for the treatment of metastatic breast carcinoma (Crown and O'Leary, 2000). Since then, paclitaxel has been used for the clinical treatment of several different cancers, as the HIV-associated Kaposi's sarcoma, small-cell lung cancer, and squamous cell cancers of the head and neck (Saville et al., 1995; Welles et al., 1998; Kim et al., 2011).

Paclitaxel is a highly hydrophobic drug (water solubility < 0.3 μg/mL). Because of its poor solubility, specific surfactants, such as Cremophor EL, a mixture of 50:50% (v/v) polyoxyethylated castor oil and absolute alcohol, are used to formulate this drug in commercial injection solutions. However, serious hypersensitivity reactions, are provoked by Cremophor EL used in the paclitaxel formulation (Fjallskog et al., 1993; Panchagnula 1998). To overcome this problem several drug delivery systems (Alkan et al., 1994; Shrama et al., 1995; Burt et al., 1999; Kan et al., 1999; Das et al., 2001; Feng and Huang, 2001) and especially liposomes (Crosasso et al., 2000) have been used. Unfortunately, the amount of paclitaxel that can be incorporated into lipid bilayers is limited (Balasubramanian and Straubinger, 1994; Shieh et al., 1997; Sharma et al., 1998). A valid approach to overcome this problem is the use of lipophilic prodrug of paclitaxel which could be retained in a lipophilic carrier. In this article, the interaction between a lipidic structure represented by a dimyristoylphosphatidylcholine (DMPC) monolayer and the lipophilic

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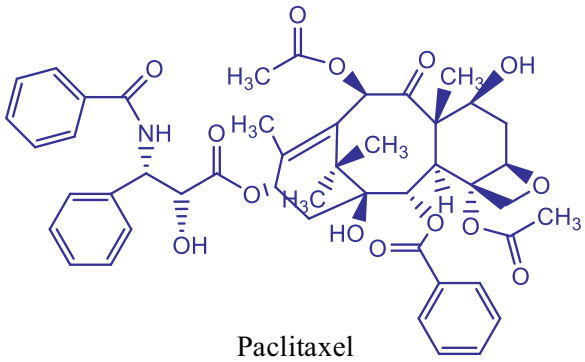
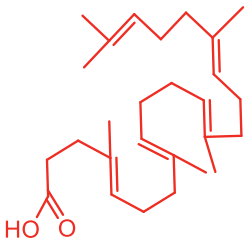




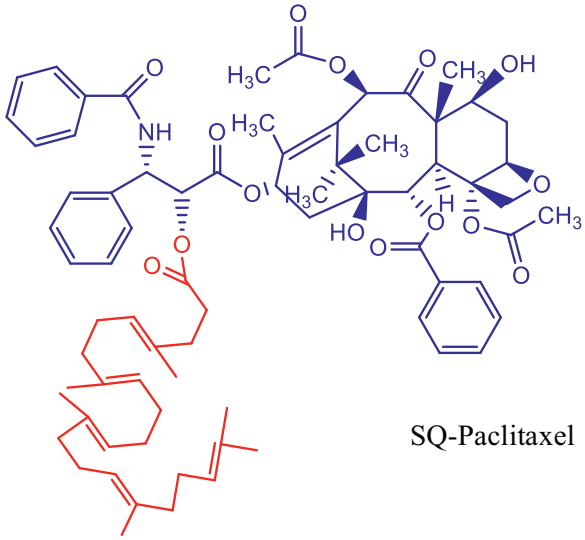


Fig. 1. Paclitaxel, SQ–COOH and SQ–paclitaxel structures.

prodrug of paclitaxel, SQ–paclitaxel, obtained by conjugation of paclitaxel with 1,1,2-trisnorsqualenoic acid (SQ–COOH) (Sarpietro et al., 2012), was studied.

Squalene is a practically water insoluble (Van Tamelen, 1968) polyunsaturated triterpene containing six isoprene units; it is the biochemical precursor of cholesterol and other steroids. In humans, squalene is synthesized in the liver and skin, it is transported into the bloodstream from VLDL (very low density lipoproteins) and LDL (low density lipoproteins), and it is secreted in large quantities by the sebaceous glands (Koivisto and Miettinen, 1998; Stewart, 1992). This triterpene is well tolerated when injected intravenously or orally administered (Gylling and Miettinen, 1994; Miettinen and Vanhanen, 1994). Principal use of parenteral administration of squalene is as vaccine adjuvant. Indeed different oil-in-water emulsion vaccine adjuvants containing squalene (named AS03, MF-59) were registered by FDA and EMA. In this field a very recent report demonstrating that squalene-based adjuvant can significantly enhance the protective efficacy of H7N9 virus vaccine appears interesting (Wu et al., 2014).

Monolayers of paclitaxel, SQ–COOH, SQ–paclitaxel and mixed monolayers of these compounds with DMPC were studied using Langmuir–Blodgett technique. The technique provides useful information on the molecular distribution, the miscibility and the interactions between molecules capable of forming monolayer (Castelli et al., 2007a,b).

2. Materials and methods

2.1. Materials

Synthetic DMPC (purity = 99.9%) was purchased from Genzyme (Switzerland). Paclitaxel was obtained from Indena (Milan, Italy). The subfase consisted of a 5 mM TRIS buffer (hydroxymethyl-aminomethane), provided by Merck and brought to pH of 7.4 by acidification with HCl (Sigma–Aldrich, Milwaukee, WI). The water used in the experiments was purified by a Milli-Q UV Plus System (Millipore Corp) and had a resistivity of 18.2 MΩ/cm.

Synthesis and characterization of SQ–paclitaxel were previously described (Sarpietro et al., 2009).

2.2. Physicochemical characterization of SQ–paclitaxel and paclitaxel

The lipophilic character of SQ–paclitaxel and paclitaxel was determined using a chromatographic R_m method (Biagi et al., 1970). Briefly, compounds were spotted on silanized silica gel TLC plates (20 × 20 cm, Bracco–Merck). The plates were developed with seven mixtures of water–methanol (from 80 to 95% of methanol) then dried and exposed to iodine vapors. The R_m were calculated from the following expression: $R_m = \log[(1/R_f) - 1]$ where R_f is the distance travelled by the compound by that of solvent front. The R_m values, plotted versus increasing concentrations of methanol, were interpolated with a straight line of equation $y = (R_m 0 + ax)$. The values reported are the mean values of 3 different chromatographic runs with correlation coefficient values ranging from 0.98 to 0.99.

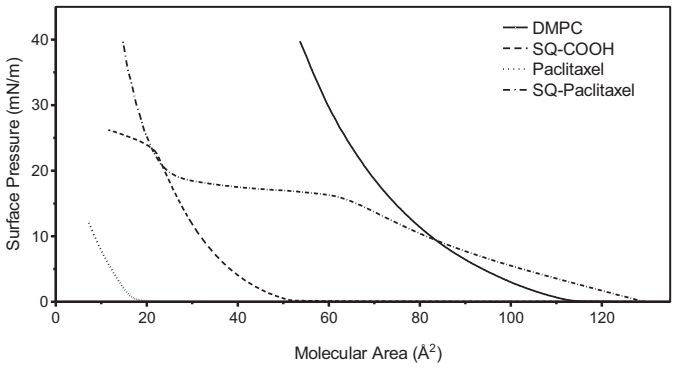
Theoretical partition-coefficient (log P) was calculated using the software ALOGPS 2.1 20 available at http://www.vcclab.org/lab/alogps/start.html,

2.3. Langmuir film balance measurements

A film balance system, KSV Langmuir minitrough (KSV, Instruments Ltd., Finland), was used. It included a Teflon 24225 mm^2 trough surrounded by a water jacket for temperature control, two mechanically coupled barriers made of Delrin. The Wilhelmy plate arrangement attached to a microbalance was used to measure the film pressure at the air/water interface. Before film deposition, the barriers were open and closed to check surface purity. The surface pressure did not differ by more than ±0.1 mN/m. The temperature of the subphase was kept constant by a thermo-stated circulating bath connected to the trough.

2.4. Molecular area/surface pressure isotherms

DMPC, SQ–eCOOH, paclitaxel and SQ–paclitaxel stock solutions in chloroform were first prepared at equimolar concentrations (0.0010 mmol/ml). Then exact volumes of phospholipids and compounds solutions were mixed to obtain the exact molar



fraction of compound with respect to DMPC (0.0; 0.03; 0.06; 0.12; 0.25; 0.5; 0.75 e 1.0). A Hamilton syringe was cleaned three times with chloroform and one with the sample; then, 30 μl of solution was drawn up and deposited drop by drop onto the aqueous surface.

After waiting for 10 min for solvent evaporation, compression was started at the speed of 10 mm/min and the $\pi - A$ isotherm was recorded. Each experiment was repeated at least three times.

2.5. Molecular area/surface pressure isotherms analysis

The molecular area as a function of the monolayer composition, at different surface pressures, was calculated. The molecular area of a two-component monolayer can be calculated by:

$$A = A_1X_1 + (1 - X_1)A_2$$

where A is the mean molecular area, X_1 is the molar fraction of component 1, $X_2 = 1 - X_1$, and A_1 and A_2 are the molecular area occupied by the pure components at the same surface pressure. Reporting in a graph A as a function of X_1 information on the two components miscibility can be obtained. If they are ideally miscible or completely immiscible at the air/liquid interface a straight line is obtained (Gaines, 1966), if the two components are not ideally miscible, deviations from the straight line are obtained.

To determine the state of the investigated monolayers, the compressibility modulus (or elasticity modulus) was calculated by the following equation:

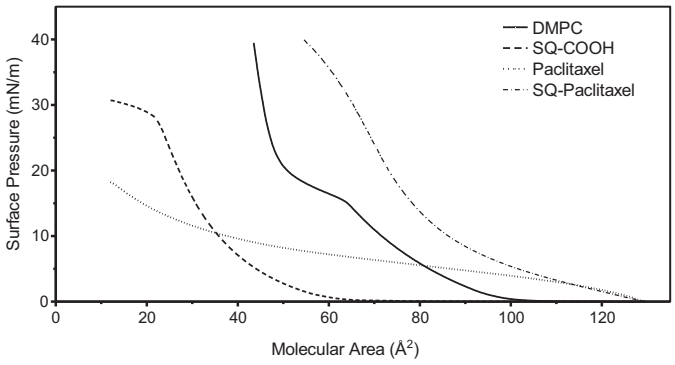
$$C_s^{-1} = -A\left(\frac{d\pi}{dA}\right)$$

where A represents the area per molecule at a given surface pressure π.

A powerful tool for evaluating the stability of a monolayer with a two or more components is excess Gibbs energy (G_{ex}). Excess free energy of mixing was calculated by applying the Eq.:

$$\Delta G_{ex} = \int_0^{\pi} [A_{12} - (X_1A_1 + X_2A_2)]\delta\pi$$

where A_1 is the area per molecule of the component 1 and X_1 its mole fractions; A_2 is the area per molecule of the component 2 and X_2 its mole fractions (Hac-Wydro and Wydro, 2007; Chimote and Banerjee, 2008). If one component is completely mixed with the other monolayer component (ideal mixing), G_{ex} attains zero value. Similarly, presence of repulsive interactions between two monolayer components results in a positive Gibbs excess, while



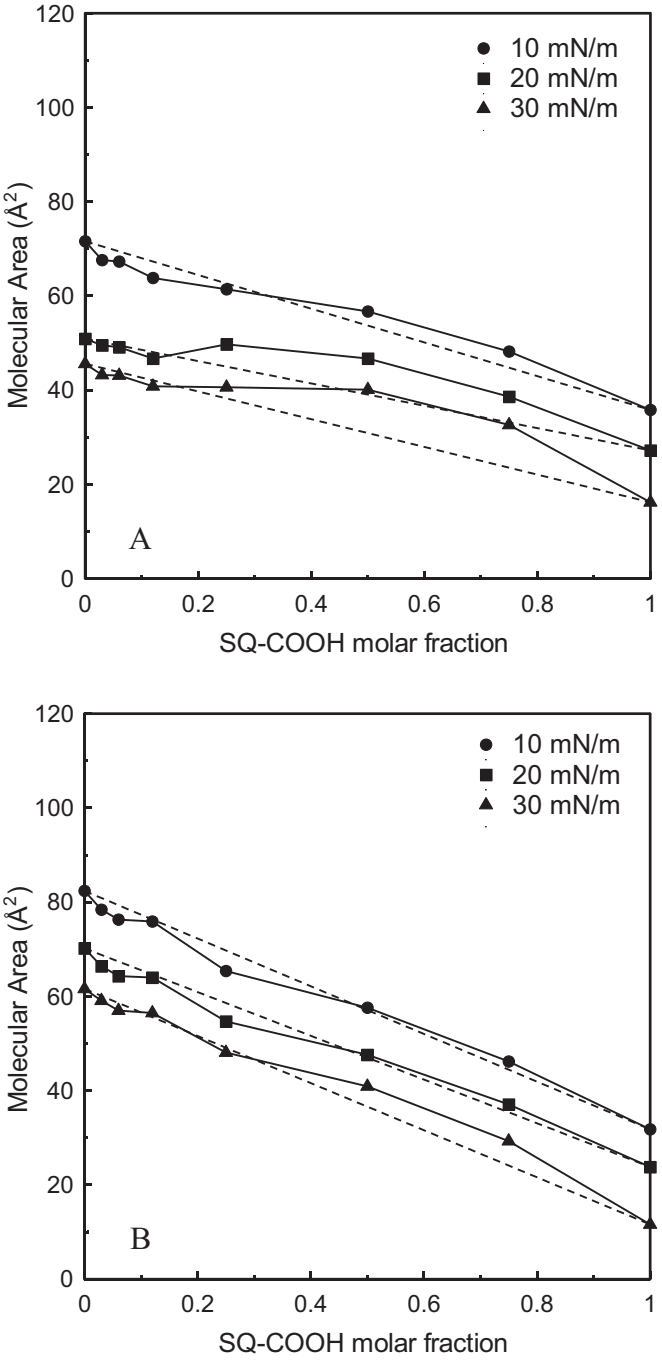


Fig. 4. Molecular area of DMPC/SQ–COOH mixed monolayers as a function of the molar fraction of SQ–COOH at the temperature of (A) 10 °C and (B) 37 °C, respectively, and at the surface pressure of 10 mN/m, 20 mN/m and 30 mN/m.

associative or attractive interactions between two monolayer components results in a negative Gibbs excess (Chou and Chang, 2000).

4. Results and discussion

4.1. Physicochemical characterization of SQ–paclitaxel and paclitaxel

The SQ–paclitaxel and paclitaxel were principally characterized by evaluating the relative lipophilicity factor (R_m), following the procedure described by Biagi and coworkers (Biagi et al., 1970) where the R_m value was extrapolated to 100% water (R_m^0). The partitioning of compounds between a non-polar stationary and a polar phase is a function of their structure in the medium and, under these conditions, it has been observed that squalene moiety dramatically increased the lipophilicity of paclitaxel. Indeed the R_m^0 of paclitaxel (8.76) increased to a value of 14.35 for SQ–paclitaxel. This experimental evaluation was confirmed by theoretical partition-coefficient values where log P moved from 3.20 for paclitaxel to 7.30 of its squalenoyl compound.

4.2. Molecular area/surface pressure isotherms

Fig. 2 shows the single compounds monolayer isotherms at 37 °C. DMPC exhibits a liquid expanded (LE) state starting from about a molecular area of 102 Å^2. SQ–COOH is in a gas-like phase up to about 52 Å^2 molecular area and a liquid expanded phase at lower value of molecular area. Paclitaxel is in a liquid expanded state starting from about 20 Å^2 molecular area and the highest surface pressure obtained is about 12 mN/m. This results agree with those reported by Zhao and Feng (2004). They explained these different behaviors considering the different natures of the compounds: DMPC and SQ–COOH are amphiphilic molecules that with their hydrophilic portion prefer to contact the water phase while their

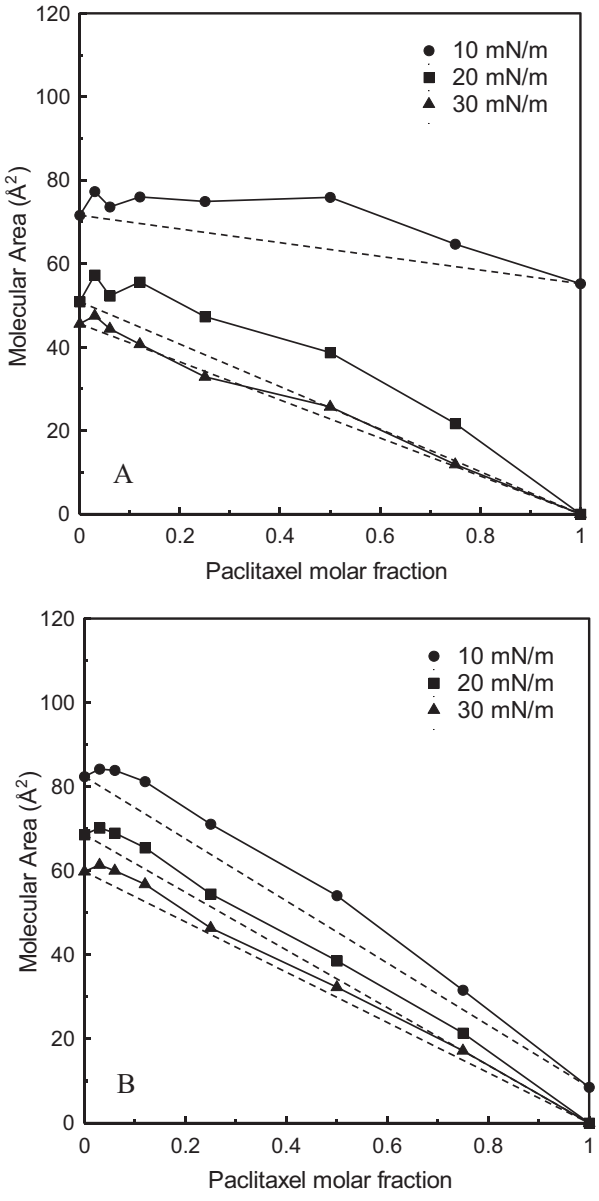


Fig. 5. Molecular area of DMPC/paclitaxel mixed monolayers as a function of the molar fraction of paclitaxel at the temperature of (A) 10 °C and (B) 37 °C, respectively, and at the surface pressure of 10 mN/m, 20 mN/m and 30 mN/m.

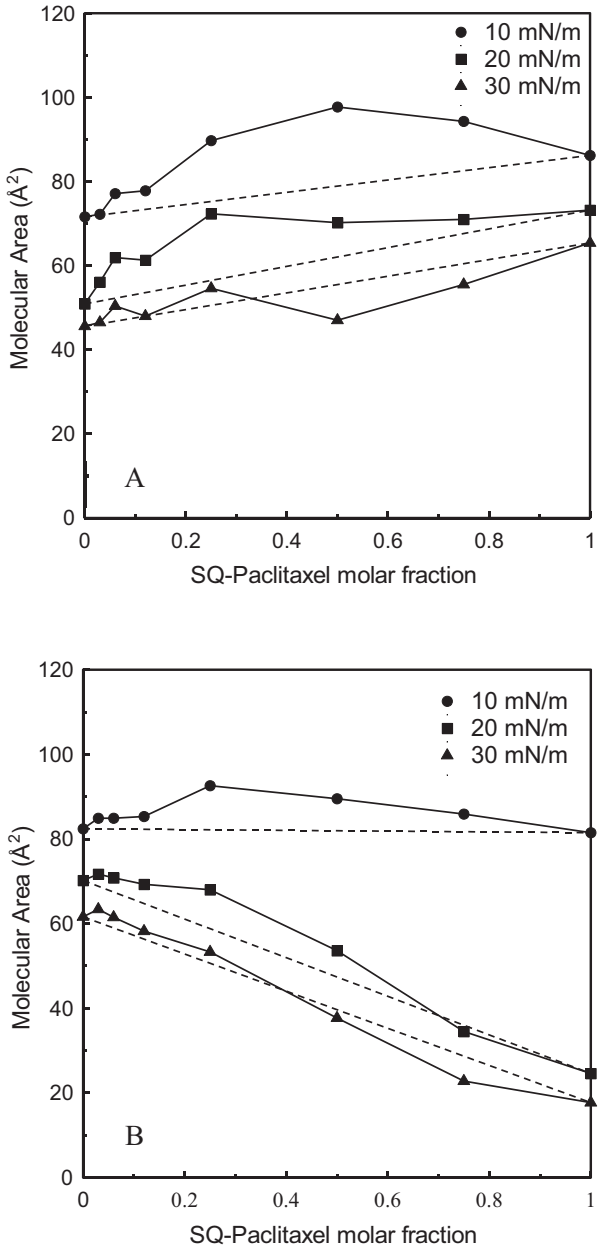


Fig. 6. Molecular area of DMPC/SQ–paclitaxel mixed monolayers as a function of the molar fraction of SQ–paclitaxel at the temperature of (A) 10 °C and (B) 37 °C, respectively, and at the surface pressure of 10 mN/m, 20 mN/m and 30 mN/m.

hydrophobic part is stretched into the air phase. This causes them to expand at the interface to lower the surface tension of the air–water interface. Instead, paclitaxel that is highly hydrophobic does not have the same tendency to spread at the air-water interface. SQ–paclitaxel isotherm indicates a liquid expanded phase up to about 75 Å^2 molecular area; then a plateau up to 25 Å^2 and a liquid condensed (LC) phase for higher values of molecular area are present. The plateau is due to phase coexistence, associated with the first-order phase transition between the LE to the LC states (Kamilya et al., 2007).

The influence of SQ–COOH on DMPC in DMPC/SQ–COOH mixed monolayers is shown in Fig. S1 (ESI). The increase of the molar fraction of SQ–COOH causes the isotherms to shift towards lower values of molecular area. Paclitaxel (Fig. S2, ESI) does not cause important variation in the isotherms up to 0.12 molar fraction; at higher molar fraction it causes a large shift towards lower molecular area. Quite different is the behaviour of SQ–paclitaxel/DMPC mixed monolayers (Fig. S3, ESI). The effect of SQ–paclitaxel on DMPC monolayers is highly dependent on its molar fraction. In fact, up to 0.12 molar fraction, the isotherms slightly shift towards higher molecular area values but maintaining almost unchanged their shape (indicative of a LE state). At higher molar fractions the mixed monolayers behave differently; in fact, below 15 mN/m the isotherms are still at higher molecular area values and reflect a LE state whereas above 15 mN/m they largely shifts towards lower molecular area values with respect to DMPC. In addition a transition from LE to LC state is present.

Fig. 3 resembles the pure compound monolayer isotherms at 10 °C. DMPC, from 100 to about 65 Å^2 molecular area is in a LE state, from 65 to about 50 Å^2 molecular area shows the transition from the LE to the LC state and from 50 Å^2 to lower values of molecular area is in a liquid-condensed state as the surface pressure increases rapidly with decreasing molecular area. Paclitaxel forms a monolayer, reaching a value of surface pressure of almost 20 mN/m. The isotherm of SQ–COOH shows that the compound is in a gaseous state up to about 60 Å^2 and from 60 up to about 25 Å^2 is in a liquid expanded state. Increasing the compression, there is not a significant increase in the surface pressure values even if the decrease of the occupied area is observed. SQ–paclitaxel occupies a greater area than that occupied by DMPC and it is in a gaseous state up to about 90 Å^2 and in a liquid expanded state for lower values of molecular area.

In SQ–COOH/DMPC mixed monolayers (Fig. S4, ESI) at 0.03 and 0.06 of SQ–COOH the isotherms do not change significantly with respect to that of DMPC and only a gradual attenuation of the LE/LC transition and a progressive displacement of isotherms towards lowest values of molecular area are visible. The LE/LC transition disappears starting from 0.25 molar fraction of SQ-COOH. At 0.5 and 0.75 molar fraction, for values of molecular area below 50 Å^2, the surface pressure increases slowly with a behavior similar to that observed for SQ–COOH.

The addition of paclitaxel to DMPC monolayers (Fig. S5, ESI) causes variations in the isotherm. For the molar fractions 0.03–0.06 the isotherms shift to higher molecular area values. The LE/LC transition moves towards slightly higher values of surface pressure and become less noticeable. The isotherms related to 0.5 and 0.75 molar fractions are in a gaseous state up to about 120 Å^2 and in a liquid expanded state at lower values of molecular area. The shape of the isotherms for these two molar fractions is similar to that of the compound.

In the isotherms of SQ–paclitaxel/DMPC mixed monolayers (Fig. S6, ESI), starting from the lowest molar fraction the LE/LC transition moves to higher values of surface pressure than that of DMPC and becomes less evident. From 0.03 molar fraction the isotherms progressively occupy a greater molecular area than that of DMPC.

4.3. Surface pressure/molecular area isotherms analysis

In order to obtain more information on the intermolecular interactions that are established in the mixed monolayers of DMPC/tested compounds, we report in a graph the mean molecular area as a function of the molar fractions of tested compound at different values of surface pressure (10 mN/m;

Table 1
Excess Gibbs free energies of mixing in DMPC/SQ–COOH, DMPC/paclitaxel and DMPC/SQ–paclitaxel mixed monolayers as a function of the molar fraction of compound at 10 °C and 37 °C, and at the surface pressure of 10 mN/m, 20 mN/m and 30 mN/m.

		10 °C			37 °C		
	Molar fraction	10 mN/m	20 mN/m	30 mN/m	10 mN/m	20 mN/m	30 mN/m
SQ–COOH	0.00	0.0	0.0	0.0	0.0	0.0	0.0
	0.03	−176.2	−82.9	−274.2	−149.4	−290.0	−180.6
	0.06	−129.5	−45.5	−132.9	−184.5	−375.2	−289.0
	0.12	−211.0	−163.3	−229.8	−25.7	−76.1	162.5
	0.25	−75.2	569.0	424.5	−261.9	−469.7	−180.6
	0.5	180.6	921.3	1662.0	30.1	72.2	776.8
	0.75	207.7	659.4	1634.9	105.3	204.7	939.4
	1.00	0.0	0.0	0.0	0.0	0.0	0.0
Paclitaxel	0.00	0.0	0.0	0.0	0.0	0.0	0.0
	0.03	372.8	954.7	590.4	241.9	440.5	613.1
	0.06	179.7	536.4	277.4	357.3	543.9	684.3
	0.12	383.4	1301.7	103.3	461.7	618.1	754.4
	0.25	445.6	1099.0	-234.8	432.0	367.3	280.0
	0.5	752.7	1595.8	523.9	520.9	517.8	433.5
	0.75	325.1	1080.9	90.3	278.5	511.8	406.4
	1.00	0.0	0.0	0.0	0.0	0.0	0.0
SQ–paclitaxel	0.00	0.0	0.0	0.0	0.0	0.0	0.0
	0.03	9.7	545.7	55.2	152.1	345.4	563.1
	0.06	278.4	1163.6	652.5	153.8	401.7	457.7
	0.12	267.8	930.2	4.3	181.1	550.6	337.4
	0.25	870.1	1905.9	731.6	627.7	1108.0	483.2
	0.5	1132.1	981.5	−1535.6	454.6	746.7	−352.2
	0.75	707.5	406.4	−894.2	251.4	−180.6	−1061.3
	1.00	0.0	0.0	0.00	0.0	0.0	0.0

20 mN/m and 30 mN/m). The dotted straight line shows the values of a two-component monolayer with ideal behavior obtained by plotting the values of the molecular area of pure DMPC and pure tested compound (molar fraction 1) at the values of surface pressure considered. Fig. 4A and B shows the molecular area of DMPC/SQ–COOH mixed monolayers as a function of the molar fraction of SQ–COOH at the temperature of 10 °C and 37 °C, respectively, and at the surface pressure of 10 mN/m, 20 mN/m and 30 mN/m. For low molar fractions of SQ–COOH, at both 10 and 37 °C, there is a small negative deviations with respect to the straight line, due to attractive forces between the SQ–COOH and the DMPC probably caused by the formation of hydrogen bonds between the carboxyl group of squalene and the polar head of DMPC that would keep close the molecules involved in the binding. At higher molar fractions a positive deviation is observed, especially at 20 mN/m and 30 mN/m and at the temperature of 10 °C. Fig. 5A and B shows the molecular area of the DMPC/paclitaxel mixed monolayers as a function of the molar fraction of paclitaxel at three different surface pressures (10 mN/m, 20 mN/m and 30 mN/m) recorded at 10 °C and 37 °C. At 10 °C, as a general trend, paclitaxel causes positive deviations with respect to the ideal values. At 30 mN/m and at higher molar fractions (0.05 and 0.075) a negative deviation is present. At 37 °C, paclitaxel, already at the lowest molar fractions, causes positive deviations that indicate slight repulsive interactions between the molecules of the drug and those of DMPC forming the monolayer. These positive deviations are more noticeable at 10 mN/m.

Fig. 6A and B shows the molecular area of the DMPC/SQ–paclitaxel mixed monolayers as a function of the molar fraction of SQ–paclitaxel at 10 °C and 37° C. At 10 °C, the prodrug causes positive deviations, with respect to the reference straight line, that are more evident for the higher molar fractions and the surface pressure of 10 mN/m. A similar behavior is observed at 20 mN/m although from the molar fraction 0.25 there is a decrease in the values of molecular area. At 30 mN/m, for higher molar fractions there are negative deviations. A similar behavior is observed at 37 °C although the deviation from the ideal straight line is less evident than that at 10 °C.

If among the components of a monolayer there are not specific interactions and the components form an ideal mixture the excess Gibbs energy equals zero and the average area per molecule in the mixture is the sum of the areas occupied by each component. Deviations from ideal behavior give ΔG values different than zero. Positive values of the ΔG_{ex} indicate that the interactions in the mixed film are less attractive or more repulsive and indicate the tendency of individual component molecules to interact preferentially with molecules of the same kind. Instead negative values of ΔG_{ex} indicate that the interactions between molecules are more attractive or less repulsive as compared to those of their respective pure films. It is seen that, as a general trend, for the highest molar fractions, at both 10 °C and 37 °C, the ΔG_{ex} values are positive in a wide range of the monolayer composition (Table 1, Figs. S7–S12, ESI). This means that the interactions between DMPC and the compounds are more repulsive and there is a tendency of the molecules to interact preferentially with molecules of the same kind. An evident exception is present in the DMPC/SQ–paclitaxel mixed monolayer at molar fraction 0.5 at 10 °C and 0.75 at 37 °C, at 30 mN/m, the negative values of the excess free energy of mixing could be a consequence of the more favorable packing of the molecules in the mixed monolayers than in the respective one-component films.

Usually, variations of compressibility can be correlated with phase changes in the monolayer (Gaines, 1966). This value depends on the state in which the film is organized; in fact, the higher the value, the more rigid and less compressible the monolayer. Reduction of compressibility modulus is indicative of a fluid film; in fact, C_s^{-1} value gives information about the compactness and packing of the film (Li et al., 2007; Gzyl-Malcher et al., 2011). Davies and Rideal (1963) gave the following ranges for different states of a monolayer: for an ideal (gaseous) monolayer the surface compressional modulus is equal to the surface pressure, for LE and LC state 12.5–250 and for solids 1000–2000 and the obtained values are in agreement with other literature data (Prince and Sears, 2012). A decrease of the compressibility modulus and a consequent formation of a more fluid monolayer is due to the presence of the compounds in the phospholipid monolayer especially for the prodrug SQ–paclitaxel (Fig. 7A–C). Sarpietro el

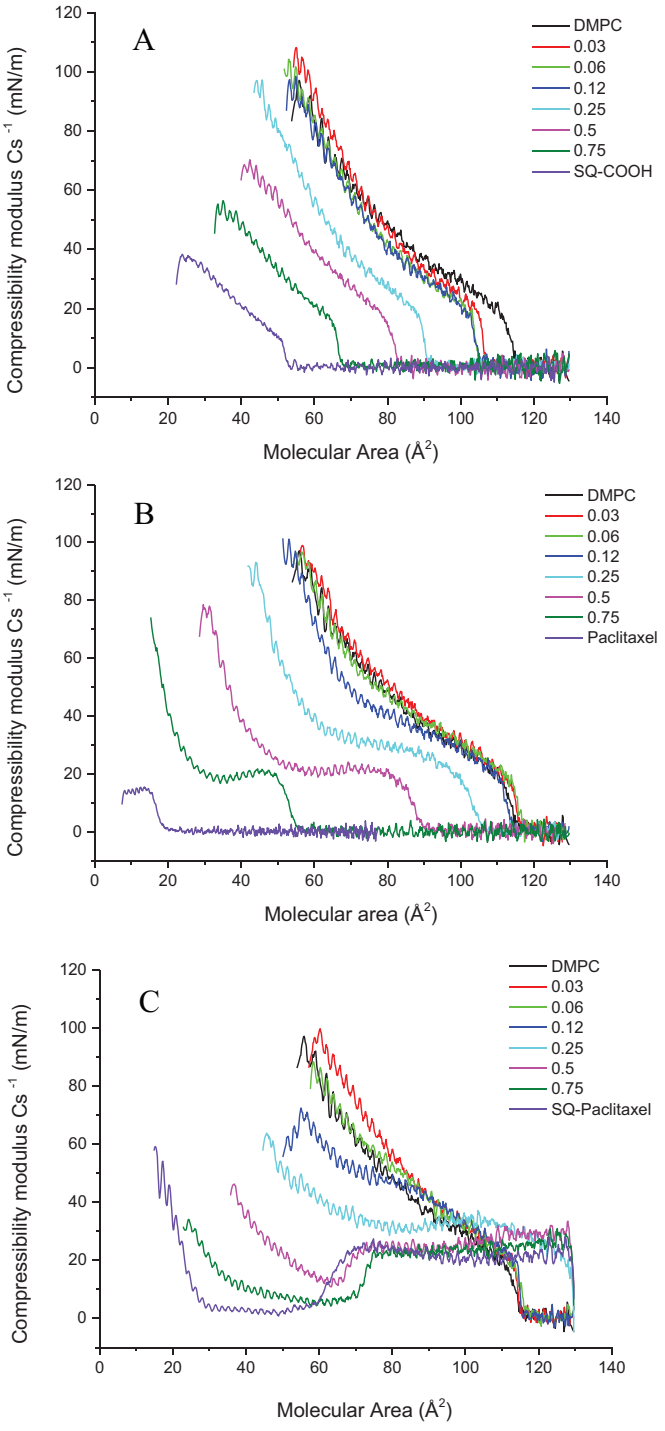


Fig. 7. Compressibility modulus–molecular area curves of DMPC/SQ–COOH (A), DMPC/paclitaxel (B), and DMPC/SQ–paclitaxel (C) monolayers at 37 °C.

al. (2012) suggested that the stronger interaction of SQ–paclitaxel/DMPC with respect to paclitaxel/DMPC could be due to its increased lipophilicity that increases the affinity for the phospholipid bilayers (in the case of calorimetric measurements) or monolayers as shown in our work. This behavior could be due to the inclusion of squalene moiety among the phospholipid molecules (Sarpietro et al., 2011).

From these results a possible localization of SQ–COOH, paclitaxel and SQ–paclitaxel in the DMPC monolayer can be hypothesized. SQ–COOH caused small deviation from the straight line; then, as already suggested (Castelli et al., 2007b), it could lie parallel to the DMPC chains with the —COOH residue immersed in the aqueous subphase. Paclitaxel caused positive deviation from the ideal line. This can be due the localization of its bulky molecules among the DMPC molecules with a consequent expanding effect that forces the molecules to occupy a larger area. A different behavior can be attributed to SQ–paclitaxel. At low surface pressure the effect of SQ–paclitaxel was comparable to that of paclitaxel, even though SQ–paclitaxel caused a bigger expanding effect. At higher surface pressure the behaviors of SQ–paclitaxel and paclitaxel were quite different. In fact, paclitaxel continued exerting its expanding effect. On the other hand SQ–paclitaxel showed a negative deviation from the ideal line suggesting the establishment of attractive interactions among the molecules. This can be explained considering that, on compression, SQ–paclitaxel molecules are forced to take a position for which squalene moiety remains among the DMPC molecules and the paclitaxel moiety, remaining anchored to the squalene moiety, protrudes out of the monolayer.

5. Conclusions

In conclusion, the interaction of paclitaxel, SQ–COOH and the prodrug SQ–paclitaxel with DMPC monolayer, studied by Langmuir–Blodgett technique, allowed to have information on the organization of the compounds in the phospholipid monolayer and to highlight the higher interaction of the prodrug SQ–paclitaxel with DMPC molecules. In addition, these findings may be of use to optimize liposomal formulations of SQ–paclitaxel prodrug.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2014.09.022.

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