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Interaction of acyclovir and its squalenoyl–acyclovir prodrug with DMPC in monolayers at the air/water interface

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ABSTRACT

Acyclovir has been conjugated to the acyclic isoprenoid chain of squalene to form the squalenoyl–acyclovir prodrug. Its interaction with biomembrane models constituted by dimyristoylphosphatidylcholine (DMPC) monolayers has been studied by employing the Langmuir–Blodgett technique. The aim of the work was to gain information on the interaction of these compounds with phospholipid membranes.

DMPC/acyclovir or squalenoyl–acyclovir prodrug mixed monolayers have been prepared at increasing molar fractions of the compound and the isotherm mean molecular area/surface pressure has been registered at 10 and 37 °C. Results reveal that the squalenoyl moiety enhances the affinity of acyclovir for the biomembrane model.

1. Introduction

Acyclovir [9-(2-hydroxyethoxymethyl)guanine] is a potent and highly selective inhibitor of the replication of herpes viruses including herpes simplex virus type 1 (HSV-I) and type 2 (HSV-2), varicella-zoster virus, and Epstein-Barr virus both in cell cultures and in animals. It is currently used as a therapeutic agent for the treatment of these viruses' infections (O'Brien and Campoli-Richards, 1989; Wagstaff et al., 1994; Kim et al., 1998; Périgaud et al., 1999). To exert its action acyclovir has to cross the cellular membrane and reach the intracellular compartment where the viruses are hosted. A useful approach to improve this transfer process could be the use of a lipophilic membrane soluble prodrug of acyclovir, and/or the inclusion of the prodrug into a carrier preparation such as liposomes. Then, the knowledge of the interaction between this prodrug and the biological membrane should be of great importance.

The lipid membranes are of highly complex structures, hence their biophysical interactions with drugs are difficult to investigate. Simplified artificial membranes systems mimicking the natural lipid membranes have been developed and used to study these interactions. Among the various models of cell membranes, lipid Langmuir monolayers are extensively used because several parameters, such as lipid composition, subphase properties, pH and temperature can be chosen to imitate real biological conditions (Kaganer et al., 1999; Brezesinski and Möhwald, 2003; Gaboriaud et al., 2005). Moreover, Langmuir monolayers offer the possibility of controlling the molecular organization in a bi-dimensional structure similar to that of the biological membranes (Maget-Dana, 1999; Dynarowicz-Latka et al., 2001). By the film-balance method the surface pressure/mean molecular area (π/Å^2) isotherm curves are easily obtained. The phospholipids are spread on an aqueous subphase to permit a monomolecular distribution. If the monolayer is subjected to an area compression, the molecules distribution is modified and the molecules are forced to go from a "gaseous" or "liquid expanded" phase at low density to a "liquid condensed" phase at a higher density and, successively, to a "solid condensed" phase (Gaines, 1966; Mohwald, 1990; Prieto et al., 1998; Krasteva et al., 2000; Vollhardt and Fainerman, 2000; Broniec et al., 2007). Eventual variations in the behavior of the isotherms of the pure phospholipid, caused by the presence of a compound dispersed among the phospholipids on the aqueous surface can indicate anomalies in the perfect miscibility of the phospholipid/compound mixtures.

In previous studies of some of us, this technique was applied to define the localization of lipophilic prodrugs of gemcitabine (Stella et al., 2004/2005) in phospholipids monolayers (Castelli et al., 2007a,b). In the present study, the interaction between a lipophilic acyclovir prodrug (squalenoyl–acyclovir), obtained by conjugation of acyclovir with the squalene acyclic isoprenoid chain

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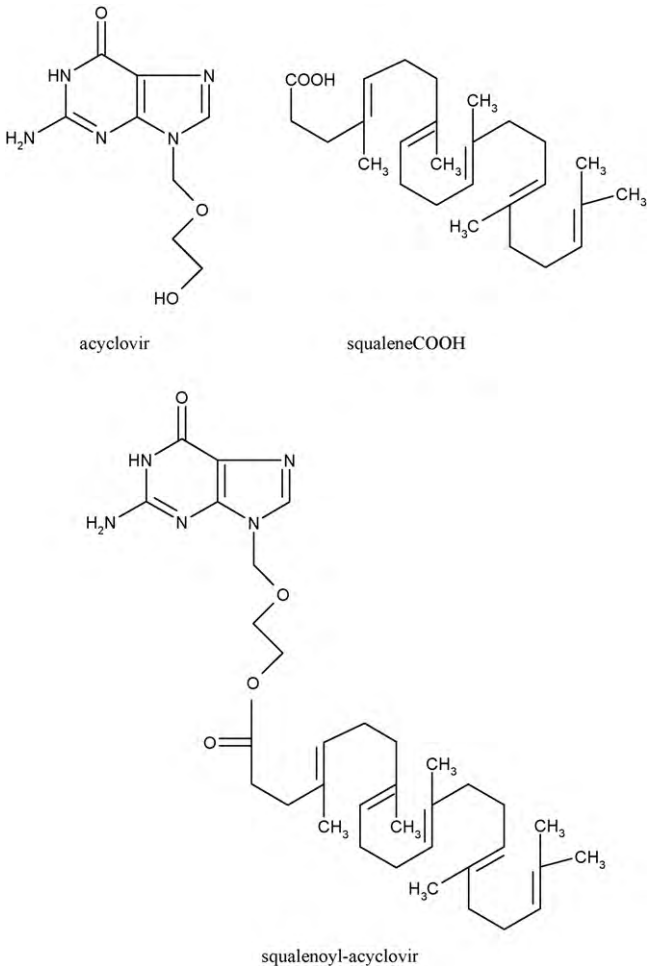

Scheme 1. Acyclovir, squaleneCOOH and squalenoyl–acyclovir structure.

(squaleneCOOH) (Scheme 1), and dimyristoylphosphatidylcholine (DMPC) in mixed monolayers was explored. To have more information, the interaction of the non-conjugate compounds (acyclovir and squaleneCOOH) with DMPC was also investigated. Results permit to obtain indication on the ability of the examined compounds to dissolve, or not, in the phospholipid molecules that form the monolayer and then to have indication on their interaction with phospholipids membranes.

2. Materials and methods

2.1. Materials

Acyclovir (purity ≥ 99%) and squalene (purity = 98%) were purchased from Sigma–Aldrich (Italy). 1,1′,2-*Tris-nor*-squalene aldehyde was obtained from squalene as previously described (Ceruti et al., 2005). Synthetic L-α-dimyristoylphosphatidylcholine (DMPC) (purity ≥ 98%) was obtained from Genzyme (Switzerland). Lipids were chromatographically pure as assessed by two-dimensional thin layer chromatography.

2.2. Synthesis of the compounds

2.2.1. 1,1′,2-Tris-nor-squalene acid: (4E,8E,12E,16E)-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenoic acid

1,1′,2-*Tris-nor*-squalene aldehyde (1.58 g, 4.11 mmol) was dissolved in diethyl ether (20 ml) at 0 °C. Separately, sulfuric acid (2.3 ml) was added at 0 °C to distilled water (20 ml) with stirring, followed by potassium dichromate (1.21 g, 4.11 mmol) to obtain chromic acid. It was then added at 0 °C within 20 min to the solution of the aldehyde previously prepared and left to react for 2 h at 0 °C with stirring. The reaction mixture was extracted with diethyl ether (50 ml 3×), washed with saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The completion of the reaction was revealed by silica gel TLC with light petroleum/diethyl ether/methanol, 70:23:7. The crude product was purified by flash chromatography with light petroleum, then light petroleum/diethyl ether, 95:5 as eluant, to give 577 mg of 1,1′,2-*tris-nor*-squalene acid (35% yield), as a colourless oil. ^{1}H NMR ($CDCl_3$): δ, 1.55–1.63 (m, 18H, allylic CH_3), 1.90–2.05 (m, 16H, allylic CH_2), 2.26 (t, 2H, CH_2CH_2COOH), 2.38 (t, 2H, CH_2CH_2COOH), 5.00–5.19 (m, 5H, vinylic CH), 12.20 (broad, 1H, COOH). MS (EI): *m/z* 400 (M^+, 5), 357 (3), 331 (5), 289 (3), 208 (6), 136 (3), 81 (100).

2.2.2. Squalenoyl–acyclovir: 9-[-(4E,8E,12E,16E)-4,8,13,17,21-pentamethyl-4,8,12,16,20-docosapentaenoyloxyethoxymethyl]-2-amino-1,7-dihydro-6H-purin-6-one

1,1′,2-*Tris-nor*-squalene acid (178 mg, 0.444 mmol) was dissolved in anhydrous DMF (1.5 ml) in a three-necked flask under nitrogen, with stirring, at room temperature, followed by dimethylaminopiridine chloridrate (DMAP) (×2.2; 120 mg, 0.98 mmol) in anhydrous DMF (1.5 ml). Acyclovir (100 mg, 0.444 mmol) was dissolved in anhydrous DMF (2 ml), with soft heating and slowly added to the previously prepared solution heated at 60 °C in a silicon bath, with stirring. When the reaction mixture resulted homogeneous, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCA) (×2.2; 188 mg, 0.98 mmol), dissolved in anhydrous DMF (1.5 ml) was added and allowed to react for 3 days, with stirring, under nitrogen, at 70 °C. The reaction mixture was controlled by silica gel TLC with ethanol/chloroform/cyclohexane, 50:25:25, transferred in a one-necked flask and evaporated to dryness. The crude derivative was dissolved in dichloromethane, washed with a 3% aqueous HCl solution, washed with saturated brine until neutral pH, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The crude product was purified by silica gel flash chromatography previously eluded alone with dichloromethane/triethylamine, 99:1 and neutralized with dicloromethane. The elution of the crude product was performed with dichloromethane/ethanol, 95:5 to give 115 mg of squalenoyl–acyclovir (42% yield), as a pale yellow viscous oil. It was completely pure, as revealed by ^{1}H NMR and mass analysis. Concerning the possibility of obtaining either an ester or an amide linkage between the squalenoyl chain and acyclovir, ^{1}H NMR analysis revealed the presence of the free amino group at 6.57 δ (2H, s, NH_2), while the signal at about 12 δ of the hypothetic amidic group was completely absent. It was also confirmed by comparison of ^{1}H NMR spectra of ester and amide acetyl derivatives of acyclovir reported in literature (Matsumoto et al., 1987). ^{1}H NMR (DMSO): δ, 1.52–1.65 (18H, m, allylic CH_3), 1.94–2.10 (16H, m, allylic CH_2), 2.13 (2H, t, $OCOCH_2CH_2$), 2.31 (2H, t, $OCOCH_2$), 3.63 (2H, m, 3′-OCH_2), 4.06 (2H, m, 4′-CH_2OCO), 5.03–5.25 (5H, m, vinylic CH), 5.32 (2H, s, 1′-NCH_2O), 6.57 (2H, s, NH_2), 7.78 (1H, s, 8-CH), 10.69 (1H, s, 1-NHCO). MS (CI): *m/z* 608 (M^+, 100).

2.3. Surface tension measurements

Film-balance measurements were performed using a KSV mini-trough apparatus provided with a computer interface unit and an operating software. The trough (24,225 mm^2 available area) made in Teflon was connected to a circulating water bath to keep the temperature constant. 5 mM Tris (pH = 7.4) in ultrapure Millipore water with resistivity of 18.2 MΩ cm was used as subphase. Equimolar solutions (0.001 mmol/ml) of DMPC, squaleneCOOH, squalenoyl–acyclovir and acyclovir in organic sol-

vents were prepared. Mixed DMPC/compound solutions were successively prepared to obtain the following molar fractions for each compound: 0.015, 0.03, 0.045, 0.06, 0.09, 0.12, 0.25, 0.50, and 0.75. 30 μl of the mixed solutions as well as the pure components were spread drop-by-drop on the surface of the subphase by a Hamilton microsyringe (which, before use, was cleaned three times with chloroform and the examined solution) and, after waiting 15 min for solvent evaporation, the monolayers were compressed by the use of two mobile barriers made in Delrin at the constant speed of 10 mm/min. Surface pressure vs. molecular area isotherms were recorded by the Wilhelmy plate arrangement attached to a microbalance. Before spreading the sample, the subphase was checked twice by running blank experiments to be sure that no impurities were present. The experiments were performed at a subphase temperature of 10 and 37 °C. Each experiment was repeated at least three times to be sure of the reproducibility of the isotherm measurements.

2.4. Surface pressure/molecular area isotherms analysis

2.4.1. Monolayer miscibility

The surface pressure/molecular area isotherms were analyzed by calculating, at different surface pressures, the molecular area as a function of the monolayer composition (expressed in molar fraction). The averaged molecular area of a two-components 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 areas per molecule of the monolayer pure components at the same surface pressure. When the two components are either ideally miscible or completely immiscible at the air/liquid interface, reporting in a graph A as a function of X_1, a straight line is obtained (Gaines, 1966; Shahgaldian and Coleman, 2003). Any deviation from the straight line indicates that the miscibility of the components is non-ideal.

2.4.2. Monolayer stability

A convenient and powerful tool to evaluate mixed monolayer stability is excess Gibb's energy (ΔG_{ex}). In case of ideal mixing between monolayer components, where one component is completely mixed with the other monolayer component, ΔG_{ex} attains 0 value. Similarly, the presence of repulsive interactions between monolayer components results in a positive Gibb's excess, while associative or attractive interactions result in a negative Gibb's excess (Chou and Chang, 2000). Excess free energy of mixing was calculated by applying the equation: $\Delta G_{ex} \int_0^{\pi} [A_{12} - (X_1A_1 + X_2A_2)]\, d\pi$; where X_1 and X_2 are the molar fractions of the two components, while A_1 and A_2 are the molar areas of the monolayer pure components; A_{12} is the effective molar area occupied by the mixed monolayer and π is the surface pressure. Positive Gibb's excess is suggestive of the formation of thermodynamically unstable monolayers, while a negative Gibb's excess indicates stable systems (Chimote and Banerjee, 2008).

3. Results and discussion

3.1. Molecular area/surface pressure isotherms

Mixed monolayers of DMPC/acyclovir, DMPC/squaleneCOOH and DMPC/squalenoyl–acyclovir at the air/water interface have been prepared and their behavior has been studied and compared with that of single component monolayers. The molecular area/surface pressure isotherms have been recorded at 10 °C (Fig. 1A–C) and 37 °C (Fig. 2A–C), below and above the pure phospholipid transition temperature.

10 °C measurements. When distributed as a monolayer, molecules exist in different states, depending upon surface pressure and temperature. At low pressure, the DMPC monolayer is in a gaseous-like phase (the surface area per molecule ranging from 120 to about 100 Å^2) with the acyl chains most apart in the air. Further compression forces the monolayer into the liquid expanded (LE) phase (area between 100 and 65 Å^2); then the monolayer is subjected to a liquid-expanded–liquid-condensed (LE–LC) transition (area from about 65 to 45 Å^2). Finally, a further compression yields a liquid condensed (LC) phase (area lower than 45 Å^2) producing a steep rise in the surface tension.

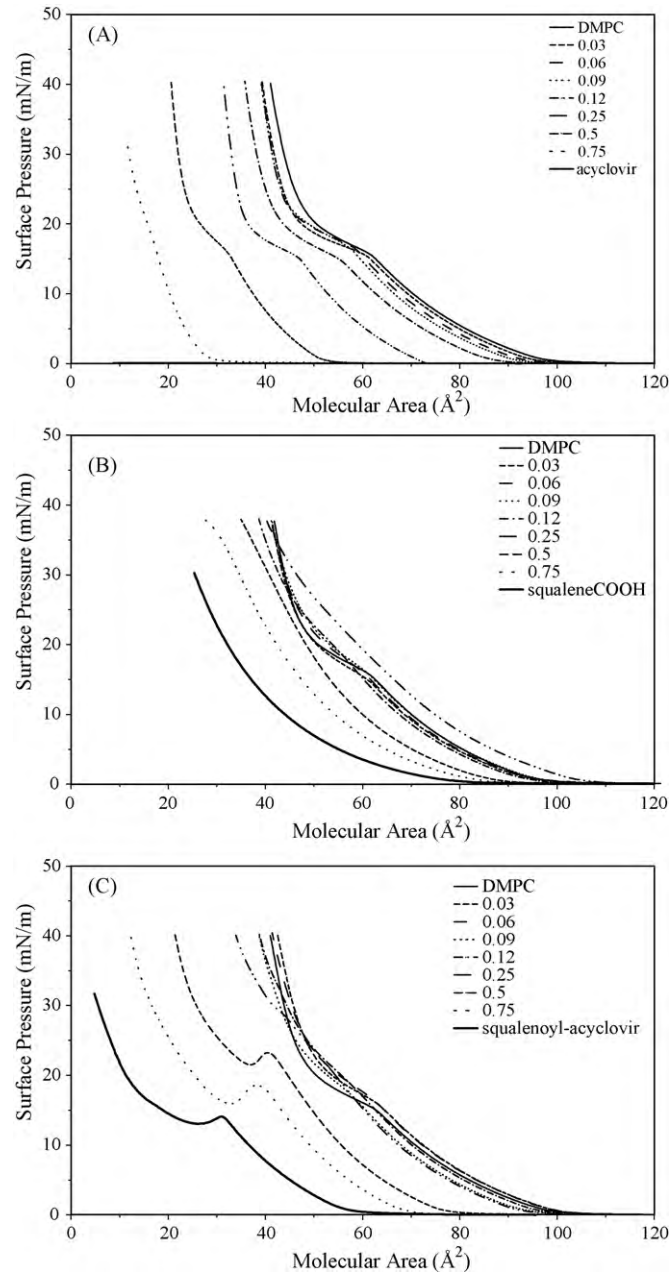

Fig. 1. Surface pressure/molecular area isotherms of DMPC and (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir mixed monolayers at the air–water interface at 10 °C.

Acyclovir does not form monolayers, but its presence, at molar fraction higher than 0.09, causes the isotherms to gradually move towards lower molecular areas and the LE–LC transition temperature to gradually decrease (Fig. 1A). SqualeneCOOH is in a gaseous state in the range of 120–85 Å^2, and in a LE state at areas smaller than 85 Å^2. This compound, at low molar fraction, does not cause

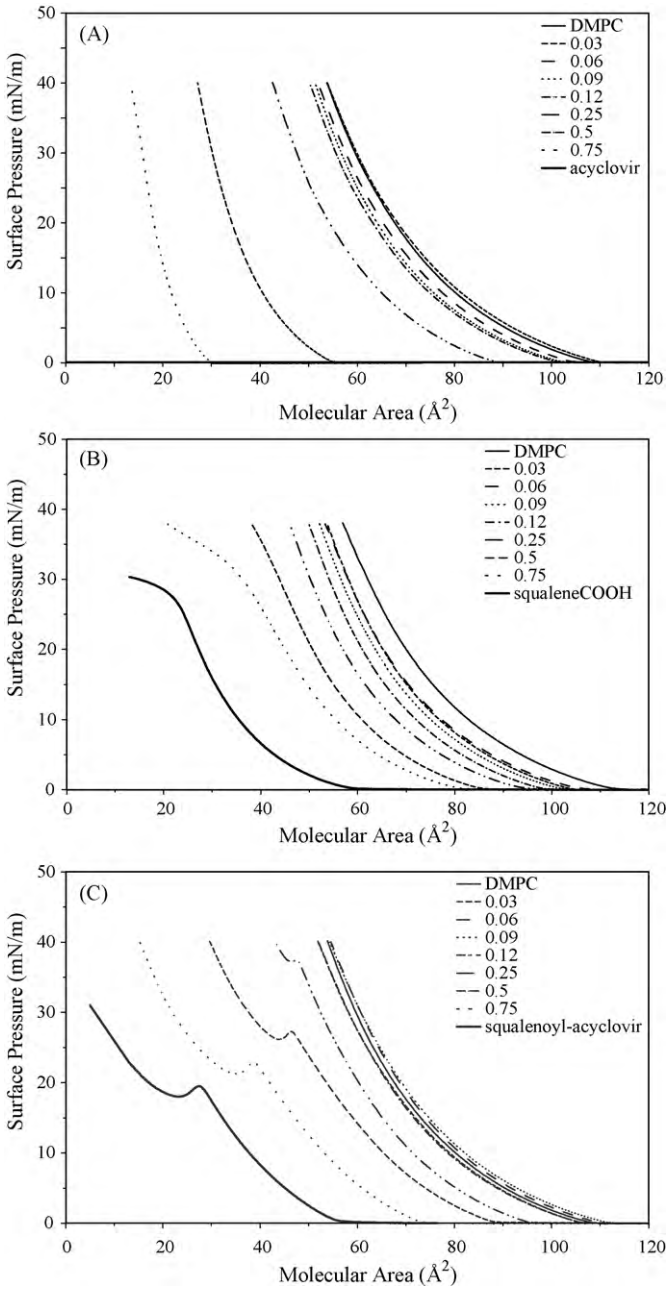

Fig. 2. Surface pressure/molecular area isotherms of DMPC and (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir mixed monolayers at the air–water interface at 37 °C.

significant variations of the isotherm: at 0.25 M fraction the LE–LC transition disappears and the isotherm moves towards higher value of the molecular area; higher squaleneCOOH molar fractions cause the isotherms to shift towards lower molecular area values (Fig. 1B).

Squalenoyl–acyclovir is characterized by a gaseous phase from 120 to 65 Å^2, a LE phase from 65 to about 35 Å^2, a LE–LC transition from about 35 to 10 Å^2, and then a LC phase. The presence of squalenoyl–acyclovir, up to 0.09 M fraction, does not determine substantial variations of the DMPC isotherms, just a small shift towards higher molecular areas; at 0.12 M fraction, the isotherms lose the LE–LC transition, although their shape remains almost unchanged. As the squalenoyl–acyclovir molar fraction further increases, the isotherms move towards lower molecular areas and exhibit a well-defined transition (Fig. 1C).

37 °C measurements. DMPC shows two regions: a gaseous phase from 120 to 110 Å^2 and a LE region starting from 110 Å^2. Even at this temperature, acyclovir does not show any isotherm. Up to 0.03 M fraction, acyclovir causes the isotherm to move towards higher molecular area, but at higher molar fraction, it causes the isotherm to move towards lower molecular area (Fig. 2A). SqualeneCOOH is

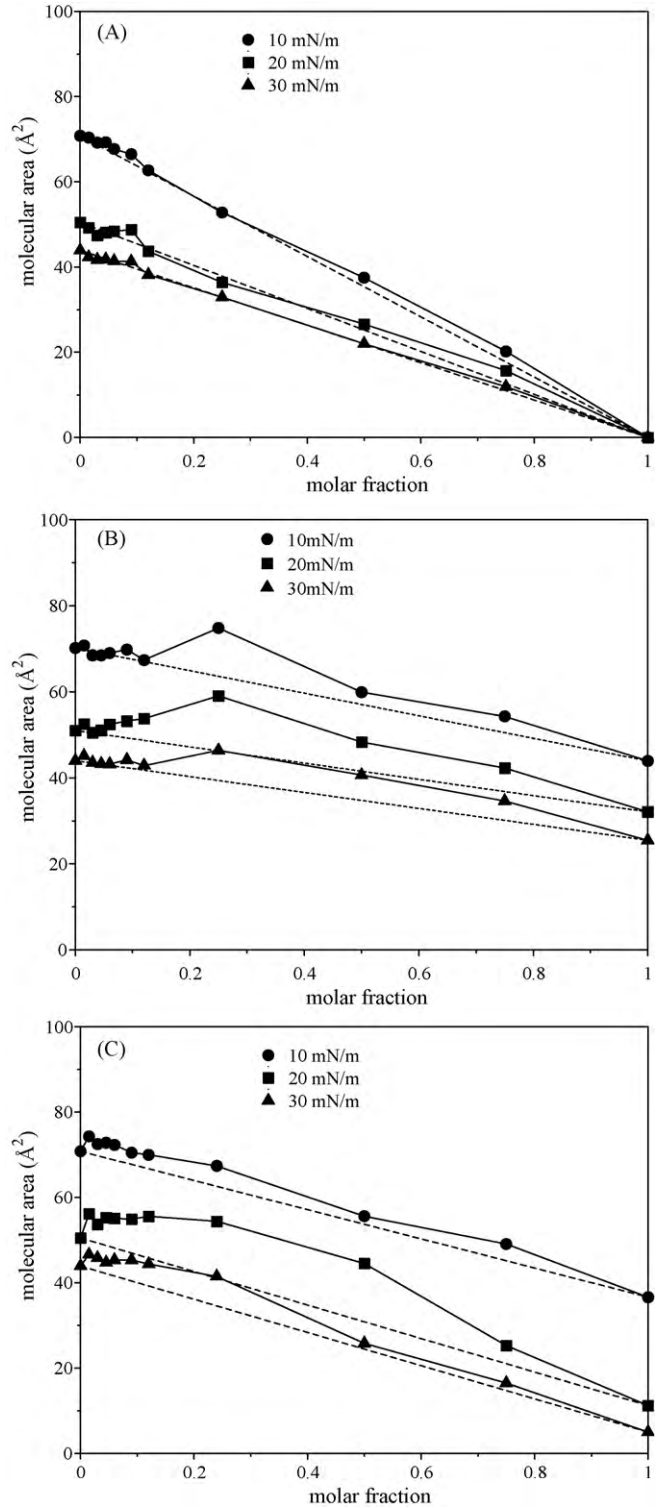

Fig. 3. Molecular area of the mixed monolayers of DMPC and (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir at the air–water interface plotted as a function of the molar fraction of compound at various values of surface pressures at 10 °C.

in a gaseous state at molecular areas between 120 and 60 Å^2, and in a LE state at lower areas. The increase of squaleneCOOH molar fraction in DMPC monolayers determines the isotherm to gradually shift towards smaller areas (Fig. 2B). Even at 37 °C, as the compression increases, squalenoyl–acyclovir presents four regions: a gaseous phase from 120 to 60 Å^2, a LE phase from 60 to about 30 Å^2, a LE–LC transition from about 30 to 15 Å^2, and a LC phase for smaller molecular areas. No significant variations are noted in the isotherms with squalenoyl–acyclovir up to 0.12 M fraction; at higher molar fractions, squalenoyl–acyclovir, causes the isotherms to shift towards lower molecular areas and the LE–LC transition to appear and became more evident (Fig. 2C)

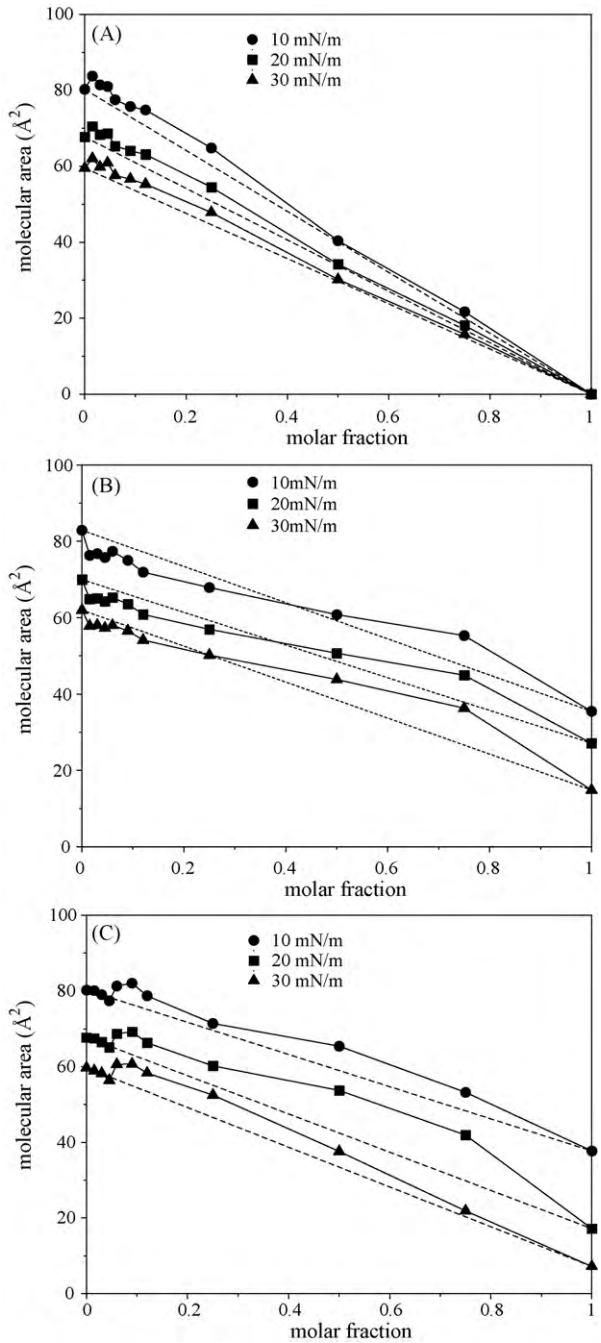

Fig. 4. Molecular area of the mixed monolayers of DMPC and (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir at the air–water interface plotted as a function of the molar fraction of compound at various values of surface pressures at 37 °C.

3.2. Surface pressure/molecular area isotherms analysis

3.2.1. Monolayer miscibility

The mean molecular area of the mixed monolayers has been reported as a function of compound molar fraction at 10, 20 and 30 mN/m (Figs. 3A–C and 4A–C).

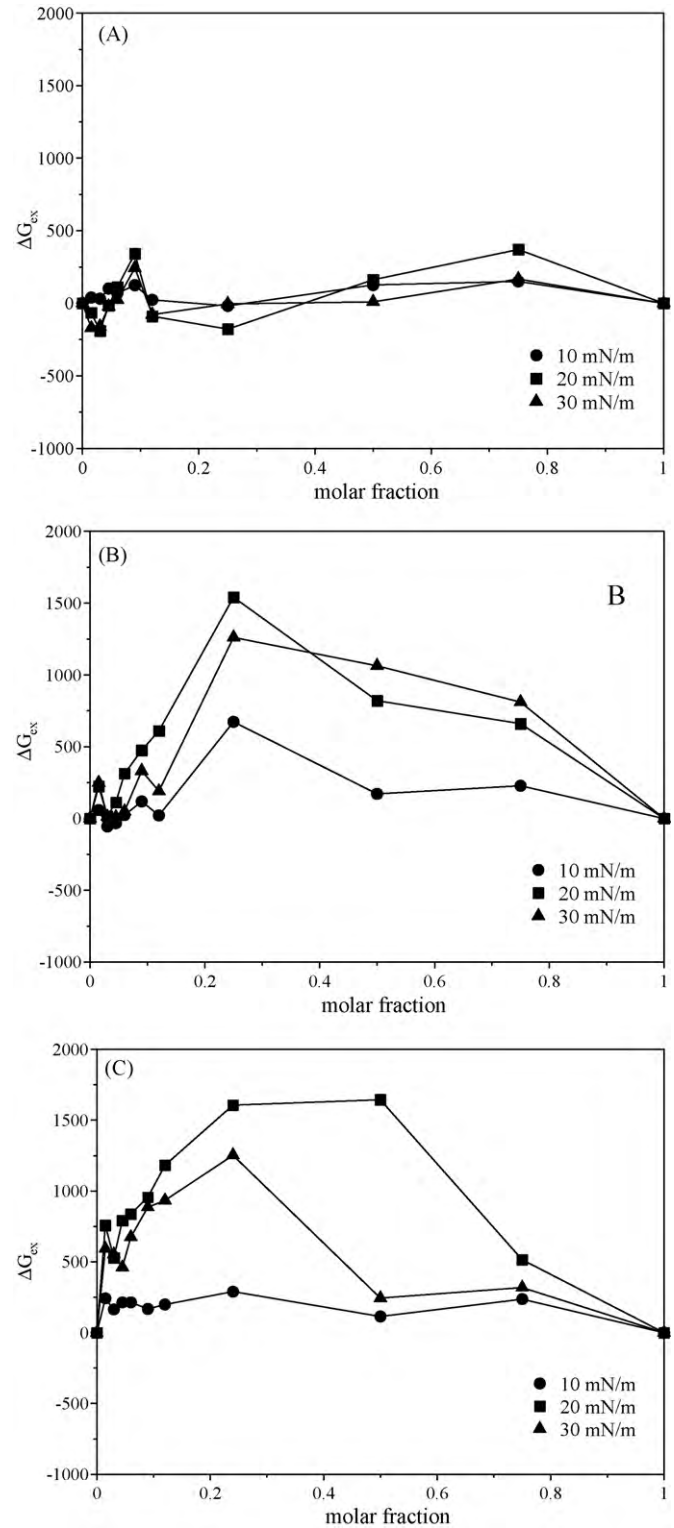

Fig. 5. Excess Gibb's free energies of mixing in DMPC and (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir mixed monolayer as a function of the molar fraction of compound at various values of surface pressures at 10 °C.

10 °C measurements. With regard to acyclovir/DMPC mixed monolayers (Fig. 3B), at low molar fraction of acyclovir, the experimental values almost coincide with the ideal values, suggesting good miscibility between acyclovir and DMPC; at high acyclovir molar fraction, a positive deviation is found, suggesting the formation of a non-ideal monolayer with dominant repulsive interactions. In squaleneCOOH/DMPC mixed monolayers (Fig. 3B), at low squaleneCOOH molar fractions, the experimental values overlap the ideal behavior, suggesting a complete and the ideal miscibility between the monolayer components. At high squaleneCOOH molar fractions, positive deviations are present indicating that repulsive interactions occur among squaleneCOOH and DMPC molecules. The behavior of squalenoyl–acyclovir/DMPC mixed monolayers positively deviate from the ideality at all the squalenoyl–acyclovir molar fractions and surface pressures, suggesting repulsive interactions between the monolayer components (Fig. 3C).

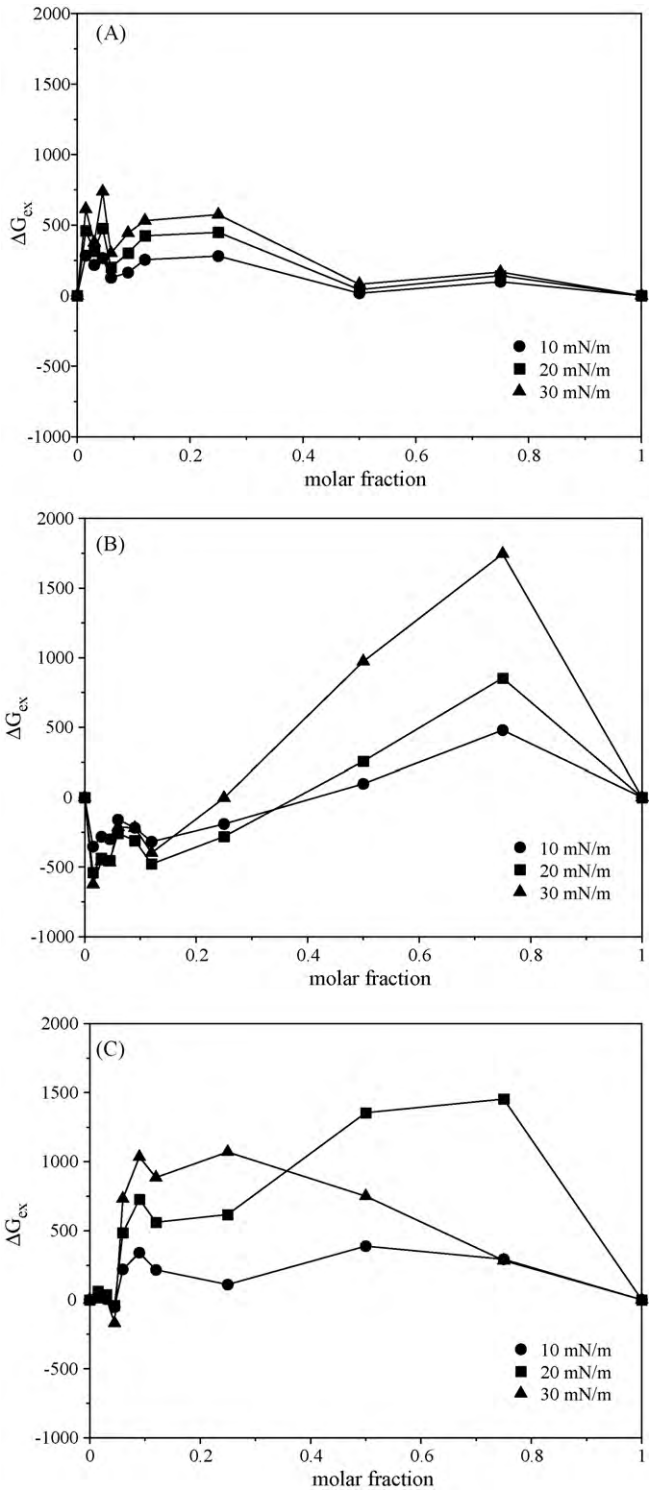

Fig. 6. Excess Gibb's free energies of mixing in DMPC and (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir mixed monolayer as a function of the molar fraction of compound at various values of surface pressures at 37 °C.

37 °C measurements. Positive deviations, and hence repulsive interactions, are observed in acyclovir/DMPC mixed monolayers for all the acyclovir molar fractions and surface pressures, particularly at 20 mN/m (Fig. 4A). Increasing the squaleneCOOH molar fraction, the squaleneCOOH/DMPC mixed monolayers exhibit first negative deviations, then, positive deviations for all the considered surface pressures. It suggests the formation of immiscible monolayers where attractive and repulsive interactions occur at low and high squaleneCOOH molar fraction, respectively (Fig. 4B). Squalenoyl–acyclovir mixed monolayers, up to 0.045 M fraction of squalenoyl–acyclovir, show an ideal behavior. On increasing the squalenoyl–acyclovir molar fraction, especially at 20 mN/m, the occurrence of positive deviations is suggestive of repulsive forces between DMPC and squalenoyl–acyclovir (Fig. 4C).

3.2.2. Monolayer stability

10 °C measurements. The behavior of ΔG_{ex} against the acyclovir molar fraction is shown in Fig. 5A. On increasing the acyclovir concentration, negative and positive deviations of ΔG_{ex} alternate, suggesting stabilization and de-stabilization of the monolayer with acyclovir concentration. With regard to squaleneCOOH (Fig. 5B), generally positive deviations are found, indicating the formation of squaleneCOOH/DMPC unstable monolayers. In the case of squalenoyl–acyclovir (Fig. 5C), at all prodrug molar fractions, positive deviations are present suggesting unstable squalenoyl–acyclovir/DMPC monolayers.

37 °C measurements. Acyclovir (Fig. 6A) causes positive deviation in the acyclovir/DMPC monolayer, especially at low molar fractions, leading to the formation of unstable monolayers. At low molar fraction, squaleneCOOH (Fig. 6B) causes negative deviations, becoming positive at high molar fractions. This suggests that at low molar fraction of compound, a stable monolayer exists, which becomes unstable by increasing the squaleneCOOH molar fraction. In the squalenoyl–acyclovir/DMPC monolayers (Fig. 6C), the experimental values overlap the ideal values at very low molar fractions of squalenoyl–acyclovir, then strong positive deviations (unstable monolayers) are observed.

4. Conclusion

The lipophilic squalenoyl–acyclovir prodrug was obtained by conjugation of acyclovir with the squalene acyclic isoprenoid chain. The behavior of the prodrug, acyclovir and squaleneCOOH in the monolayer at the air/water interface and their interaction with a biomembrane model made up of dimyristoylphosphatidylcholine monolayers was studied. The above reported Langmuir–Blodgett measurements give information not obtainable by other techniques, evidencing that the squaleneCOOH group confers to the prodrug a behavior quite different than that shown by acyclovir. In particular, acyclovir does not form any monolayer, while the prodrug forms a stable monolayer which evidence a characteristic phase transition upon compression. This transition is preserved in DMPC/squalenoyl–acyclovir mixed monolayers at high prodrug molar fractions. All the studied compounds interact with DMPC, as also reported in previous studies carried out by differential scanning calorimetry of mixed multilamellar vesicles (Sarpietro

et al., 2009). The different effect of the investigated compounds on DMPC monolayer is considerably influenced by the presence of the squalenoyl group bound to acyclovir which modifies the shape of the isotherms. These effects are much more evident than those previously observed by calorimetry (Sarpietro et al., 2009). In particular, the positive deviations of both the surface area excess (Figs. 3 and 4) and the Gibb's free energy excess (Figs. 5 and 6) clearly indicate repulsive interactions between the monolayer components. Furthermore, the comparison of all the above results may provide information on the localization of the three compounds in DMPC monolayers. Acyclovir produces a small expansion effect, probably because it is localized near the DMPC polar heads and do not contribute to the hydrophobic interactions among the phospholipid chains. SqualeneCOOH could be localized parallel to the phospholipids chain with the carboxylic group protruding towards the subphase. The increase of the lipophilic character of acyclovir through its conjugation to squaleneCOOH and the formation of the squalenoyl–acyclovir prodrug gives rise to a stronger interaction with DMPC monolayer with respect to those of the free drug. This effect probably arises because the prodrug molecules is inserted among the DMPC acylic chain in a way that the phospholipids molecules are forced to occupy a larger area. Almost the ideal behavior observed at 37 °C and at low molar fraction could depend on the flexibility of the molecule at this temperature, which well adapts to the surrounding phospholipid chains.

Acknowledgments

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References

Brezesinski, G., Möhwald, H., 2003. Langmuir monolayers to study interactions at model membrane surfaces. Adv. Colloid Interface Sci. 100–102, 563–584.

Broniec, A., Underhaug Gjerde, A., Ølmheim, A.B., Holmsen, H., 2007. Trifluoperazine causes a disturbance in glycerophospholipid monolayers containing phosphatidylserine (PS): effects of pH, acyl unsaturation, and proportion of PS. Langmuir 23, 694–699.

Castelli, F., Sarpietro, M.G., Micieli, D., Stella, B., Rocco, F., Cattel, L., 2007b. Enhancement of gemcitabine affinity for biomembranes by conjugation with squalene: differential scanning calorimetry and Langmuir–Blodgett studies using biomembrane models. J. Colloid Interfase Sci. 316, 43–52.

Castelli, F., Sarpietro, M.G., Rocco, F., Ceruti, M., Cattel, L., 2007a. Interaction of lipophilic gemcitabine prodrugs with biomembranes models studied by Langmuir–Blodgett technique. J. Colloid Interface Sci. 313, 363–368.

Ceruti, M., Balliano, G., Rocco, F., Lenhart, A., Schulz, G.E., Castelli, F., Milla, P., 2005. Synthesis and biological activity of new iodoacetamide derivatives on mutants of squalene-hopene cyclase. Lipids 40, 729–735.

Chimote, G., Banerjee, R., 2008. Molecular interactions of cord factor with dipalmitoylphosphatidylcholine monolayers: implications for lung surfactant dysfunction in pulmonary tuberculosis. Colloids Surf. B: Biointerfaces 65, 120–125.

Chou, T., Chang, C., 2000. Thermodynamic characteristics of mixed DPPC/DHDP monolayers on water and phosphate buffer subphases. Langmuir 16, 3385–3390.

Dynarowicz-Latka, P., Dhanabalan, A., Oliveira Jr., O.N., 2001. Modern physicochemical research on Langmuir monolayers. Adv. Colloid Interface Sci. 91, 221–293.

Gaboriaud, F., Volinsky, R., Bermana, A., Jelineka, R., 2005. Temperature dependence of the organization and molecular interactions within phospholipid/diacetylene Langmuir films. J. Colloid Interface Sci. 287, 191–197.

Gaines Jr., G.L., 1966. Insoluble Monolayers at Liquid–Gas Interfaces. Wiley-Interscience, New York.

Kaganer, V.M., Möhwald, H., Dutta, P., 1999. Structure and phase transitions in Langmuir monolayers. Rev. Mod. Phys. 71, 779–819.

Kim, D.-K., Lee, N., Im, G.-J., Kim, H.-T., Kim, K.H., 1998. Synthesis and evaluation of 2-amino-6-fluoro-9-(2-hydroxyethoxymethyl)purine esters as potential prodrugs of acyclovir. Bioorg. Med. Chem. 6, 2525–2530.

Krasteva, N., Vollhardt, D., Brezesinki, G., 2000. Mixed stearoyl-*rac*-glycerol/12-(hydroxy)stearoyl-*rac*-glycerol monolayers on the air/water interface: brewster angle microscopy and grazing incidence X-ray diffraction investigation. J. Phys. Chem. B 104, 8704–8711.

Maget-Dana, R., 1999. The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes. Biochim. Biophys. Acta 1462, 109–140.

Matsumoto, H., Kaneko, C., Yamada, K., Takeuchi, T., Mori, T., Mizuno, Y.R., 1987. A convenient synthesis of 9-(2-hydroxyethoxymethyl)guanine (acyclovir) and related compounds. Chem. Pharm. Bull. 36, 1153–1157.

Mohwald, H., 1990. Phospholipid and phospholipid–protein monolayers at the air/water interface. Annu. Rev. Phys. Chem. 41, 441–476.

O'Brien, J.J., Campoli-Richards, D.M., 1989. Acyclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drugs 37, 233–309.

Périgaud, C., Gosselin, G., Girardet, J.-L., Korba, B.E., Imbach, J.-L., 1999. The *S*-acyl-2-thioethyl pronucleotide approach applied to acyclovir. Part I. Synthesis and in vitro anti-hepatitis B virus activity of bis(*S*-acyl-2-thioethyl)phosphotriester derivatives of acyclovir. Antiviral Res. 40, 167–178.

Prieto, I., Camacho, L., Martin, M.T., Mobius, D., 1998. Ion interactions and electrostatic effects on TMPyP/DMPA monolayers. Langmuir 14, 1853–1860.

Sarpietro, M.G., Micieli, D., Rocco, F., Ceruti, M., Castelli, F., 2009. Conjugation of squalene to acyclovir improves the affinity for biomembrane models. Int. J. Pharm. 382, 73–79.

Shahgaldian, P., Coleman, A.W., 2003. Miscibility studies on amphiphilic calix[4]arene-natural phospholipid mixed films. Langmuir 19, 5261–5265.

Stella, B., Rocco, F., Rosilio, V., Renoir, J-M., Cattel, L., Couvreur, P. French Patent of 6/6/2004, n° 04 51365; International European Patent PCT/FR2005/050488 of 23/06/2005.

Vollhardt, D., Fainerman, V.B., 2000. Penetration of dissolved amphiphiles into two-dimensional aggregating lipid monolayers. Adv. Colloid Interface Sci. 86, 103–151.

Wagstaff, A.J., Faulds, D., Goa, K.L., 1994. Aciclovir. A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drugs 47, 153–205.