ELSEVIER

Biophysical Chemistry 95 (2002) 157–164

Biophysical Chemistry

www.elsevier.com/locate/bpc

Flunitrazepam-membrane non-specific binding and unbinding: two pathways with different energy barriers

Daniel A. García, María A. Perillo*

Cátedra de Biofísica-Química, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Velez Sarsfield 299 Córdoba (5000), Argentina

Received 7 December 2001; received in revised form 4 January 2002; accepted 8 January 2002

Abstract

The effect of molecular packing on flunitrazepam's ability to interact with bio-membranes was studied using dipalmitoylphosphatidylcholine monomolecular layers at the air–water interface as a model membrane. Flunitrazepam penetrated from the subphase into monolayers at lateral pressures below 44.8 mN/m and induced their concentration-dependent expansion. As inferred from the values of compressibility modulus, the elasticity of the liquid-condensed phase decreased in the presence of flunitrazepam. Although this drug hardly penetrated into high-packed monolayers, it was easily incorporated in the low-packed ones at an extent sufficient to reach the partition equilibrium. Below a molecular area of 75 $Å^2$, contrary to what would be expected, the drug surface concentration increased as a function of surface pressure, suggesting that after its penetration in disordered phases, it became energetically or physically trapped in newly-formed liquid condensed clusters. The phenomenon of flunitrazepam penetration and release would have different energy barriers depending on the membrane phase-state.

Keywords: Benzodiazepine; Phosphatidylcholine monolayer; Membrane packing; Release and penetration of flunitrazepam

1. Introduction

Benzodiazepines (BZDs) are drugs extensively used as anxiolytics, miorelaxants, hypnotics and anti-convulsants. Flunitrazepam (FNT) is a potent anxiolytic BZD; however, it has been mainly used as a night-time hypnotic and it has been applied to the induction of anesthesia in surgery [1].

The main pharmacological actions of BZDs are considered to be mediated by two kinds of specific receptors: an intrinsic site of the $GABA_A$ receptor in the central nervous system, which is known as the Central Benzodiazepine Receptor (CBR) [2], and a second kind of receptor called the Peripheral Benzodiazepine Receptor (PBR), which is found in several peripheral tissues [3]. However, the direct involvement of CBR or PBR has not been demonstrated for several of BZDs' effects exerted at global concentrations above their binding affinity for these receptors [4]. These evidences strongly suggest the possibility that BZDs could affect signal transduction mechanisms without involving

*Corresponding author. Tel.: +54-351-4332114; fax: +54-351-4332097.

E-mail address: mperillo@com.uncor.edu (M.A. Perillo).

0301-4622/02/$ - see front matter
PII: S0301-4622(02)00004-2

the receptor binding phenomenon but affecting membrane properties, as was demonstrated for other lipophilic substances [5].

Previous studies from our laboratory demonstrated that BZD-membrane non-specific interactions could be explained by a partition equilibrium model (unlimited incorporation of molecules) [6]. The thermodynamic result of a partition equilibrium is consistent with drug molecules accommodated between lipid molecules becoming an integral part of the membrane; this mechanism may eventually lead to the swelling of the membrane until it is converted to a non-bilayer phase [7].

We showed that BZD partitioning in the membrane increases in quantity and depth as the membrane structural order decreases. Moreover, the localization of BZDs at the phospholipid polar head group region would explain the effects of FNT on the decrement of dipalmitoylphosphatidylcholine (dpPC) vesicle size [8,9]. The presence of FNT in the membrane leads to an increase in the relative volume of the polar head group regions and to a decrease in the thermodynamic stability of the self-aggregating structure, which was forced towards an increase in its surface curvature [10]. We also demonstrated that those phenomena could be transduced into changes in the curvature of membranes from natural origin [11]. In a complex system, such as a cell, curvature changes triggered by the drug partitioning towards the plasma membrane might have been an indirect effect exerted through modifications of ionic gradients or by affecting cytoskeleton–membrane linkage. However, our results supported the idea that this changes in membrane curvature should be interpreted as a mechanism suitable to relieve tensions generated initially by the drug incorporation into the bilayer, and were considered the resultant of the dynamic interaction of many molecular fluxes leading to satisfy the spontaneous membrane curvature.

The component layers of a bilayer are under non-uniform tension becoming from the frustrated spontaneous curvature. This tension is likely to express itself in a modified partitioning of amphiphiles and possibly also in the activity of membrane-embedded integral proteins and hydrophobic molecules. The bending of a bilayer to a radius of curvature R, will give rise to an area expansion inversely related to R [12]. In turn, changes in molecular area can be thought of in terms of variations in molecular packing.

The aim of the present work was to study the effect of membrane molecular packing properties on the ability of FNT to penetrate and to remain at the water–lipid interface in order to improve our knowledge about BZD-membrane non-specific interactions. These studies were approached using Langmuir monomolecular layers at the air–water interface. This technique constitutes an informative and convenient membrane model because it permits subtle control of the membrane molecular packing [13].

2. Experimental

2.1. Materials

FNT (7-nitro-1,3-dihydro-1-methyl-5-(2-fluorophenyl)-1,4-benzodiazepin-2-one) was kindly supplied by Productos Roche (Buenos Aires, Argentina). DpPC was from Avanti Polar Lipids (Alabaster AL, USA). [^{3}H]-FNT was purchased from New England Nuclear Chemistry (Boston MA, USA). Water was bidistilled in an all glass apparatus. The other drugs and solvents used were of analytical grade.

2.2. Monomolecular layers at the air–water interface

Monomolecular layers were prepared and monitored essentially according to Perillo et al. [14]. The equipment used was a Minitrough II from KSV Instruments Ltd (Helsinki, Finland). Between 5 and 30 μl of a chloroformic solution of phospholipid was spread on an unbuffered aqueous surface; approximately 5 min were allowed for the evaporation of chloroform. Lateral surface pressure (π) was measured by the Wihelmy plate method. Reproducibility was within ± 0.001 nm^2 and ± 0.001 mN/m for the molecular area and surface pressure, respectively.

Three kinds of experiments were performed: (i) the release of FNT from the monolayer; (ii)

determination of the maximum value of lateral surface pressure (π) that allowed drug penetration in the monolayer ($\pi_{cut\text{-}off}$); and (iii) the effect of the monolayer molecular packing on FNT surface concentration (Γ). In release (i) and penetration (ii) experiments, we used a circular Teflon trough (4.5 cm diameter and 0.5 cm depth).

i In the first kind of experiment, we investigated the release of [^{3}H]-FNT, from a mixed monolayer prepared with dpPC plus [^{3}H]-FNT (200:1 molar ratio) at different initial lateral pressures (π_i), towards the subphase. The amount of [^{3}H]-FNT released was quantified by liquid scintillation counting. Aliquots of 10 μl were taken from the sub-phase at different times and were added to 2.5 ml of scintillation cocktail (25% Triton X-100 and 0.2% diphenyloxazol in toluene). Radioactivity was measured in a Rackbeta LKB 1214 scintillation counter from Wallac Oy (Turku, Finland) at a 60% counting efficiency for tritium and with a precision of 5%.

ii These experiments were performed at constant surface area, but at different π_i in order to measure the changes in π ($\Delta\pi$) induced by FNT penetration into the monolayer, after the injection of 20 μM FNT in the subphase. The values of $\Delta\pi$ were plotted against π_i and adjusted to a straight line; the maximum π allowing drug penetration ($\pi_{cut\text{-}off}$) was determined from the intersection of the line with the abscise axis.

iii The third kind of experiment was π-molecular areas isotherms; π values were measured at different molecular areas of the phospholipid in the absence or presence of FNT at different concentrations in the subphase. For these experiments, we used a rectangular trough fitted with two barriers that were moved synchronously by electronic switching. The signal corresponding to the surface area (automatically determined by the Minitrough according to the relative position of the two compression barriers) and the output from the surface pressure transducer (measured automatically by the Minitrough with a platinized Pt foil 5-mm wide×20-mm long×0.025-mm thick) were fed into a personal computer through a serial interface using a specific software. Before each experiment, the trough was rinsed and wiped with 70% ethanol and several times with bi-distilled water. The absence of surface-active compounds in the pure solvents and in the subphase solution (bi-distilled water) was checked before each run by reducing the available surface area to less than 10% of its original value after enough time was allowed for the adsorption of possible impurities that might have been present in trace amounts. The monolayer was compressed at a constant low rate of 20 mm^2/s at 28±0.5 °C. A lower compression rate (12 mm^2/s) was tested, and identical results were obtained.

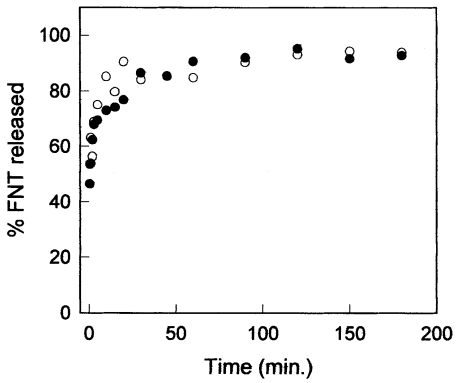

Fig. 1. FNT released from a dpPC-FNT mixed monomolecular layer to the subphase as a function of time. The values correspond to the percentage released with respect to the total amount incorporated in the monolayer, at two different surface pressures: 10 mN/m (open circle) and 30 mN/m (closed circle). The FNT released was quantified by measuring [^{3}H]-FNT as radioactive label incorporated together with FNT (rel. 1:10) in the monolayer.

From the π-area isotherms the quantity of FNT per unit area (Γ) was calculated according to the following equation derived from the Gibbs surface tension equation [15]:

$$\Gamma = \frac{c}{RT} \cdot \frac{\partial \gamma_{lip}}{\partial c} \quad (1)$$

where c is the FNT concentration in the subphase; γ_{lip} is the surface tension of dpPC monolayer (calculated from: $\gamma_{lip} = \gamma_w - \pi$, γ_w being the surface tension of water at 25 °C) for a particular

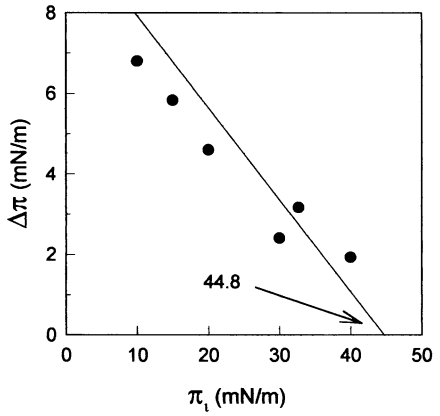

Fig. 2. Surface pressure changes ($\Delta\pi$) at different initial pressures (π_i) in the presence of 20 μM FNT. The values were adjusted to a straight line by a linear regression as was explained in Section 2, in order to determine the $\pi_{cut\text{-}off}$ value (indicated by the arrow in the graph).

molecular area. An ideal behavior of drug in solution was assumed, so the FNT activity coefficient was equaled to the units. The FNT concentration in the subphase varied from 0 to 50 μM.

3. Results and discussion

FNT was not surface active. No change in surface pressure was observed after the addition of this compound to the subphase without any lipid film on the surface (results not shown); for this reason the changes in the dpPC monolayers induced by FNT were interpreted directly as its interaction with the lipid monolayer. We chose unbuffered bi-distilled water as a subphase in order to avoid the presence of additional molecular species in the system. In spite of that, no changes in pH were expected because both the monolayer component (dpPC) and the drug had equilibrium dissociation constant values below 3. This fact assured a constant either uncharged or zwitterionic state of FNZ and dpPC, respectively, at any pH > 3. As we demonstrated previously [10], in a wide pH range between 3 and 8, the partitioning of FNZ toward dpPC bilayers remained constant.

The release of [^{3}H]-FNT from the dpPC monolayer towards the clean subphase represented the tendency of the drug to reach the equilibrium concentration between the lipidic monolayer and the aqueous subphase [16]. This release was fast and reached approximately 80% in the first 10 min. However, after 3 h, approximately 5% of the initial amount still remained in the monolayer. This behavior was similar for $\pi_i = 10$ or 30 mN/m, although at the latter π, the initial release rate was slightly faster (Fig. 1).

The $\pi_{cut\text{-}off}$ value obtained for the FNT penetration from the subphase to the dpPC monolayer was 44.8 mN/m (Fig. 2), indicating that FNT in the subphase was unable to penetrate the monolayer packed at pressures above this value.

FNT expanded the dpPC π-molecular area isotherm in a concentration-dependent manner without inducing significant changes in the shape of the control isotherm (obtained using water as a subphase) (Fig. 3a). The well known dpPC phase transition between liquid expanded (LE) and liquid condensed (LC) phase states was evident in all the isotherms obtained at different FNT concentrations but, in the presence of FNT, the phase

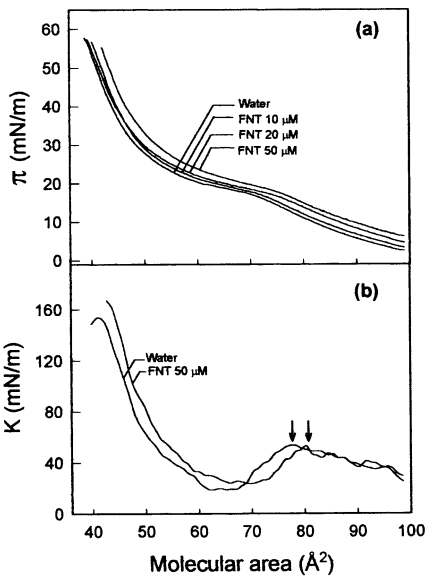

Fig. 3. Variation of surface pressure (a) and compressibility modulus (K) (b) as a function of dpPC molecular area, in the absence or in the presence of FNT at different concentrations in the subphase. The isotherms were done at 28 ± 0.5 °C. K values were calculated according to Eq. (2). The arrows in (b) approximately indicate the phase transition point.

transition occurred in higher areas than in its absence. Although the decrease in the collapse pressure of dpPC monolayers induced by FNT was not quantitatively important, the minimum molecular area suffered a noticeable increment of approximately 10% in the presence of FNT compared with the control.

Since FNT added to the subphase became from ethanolic solutions, some isotherms were done in the presence of ethanol in the subphase at final concentrations equivalent to those added as FNT solvent (0.04, 0.08 and 0.2%, v/v). In all the cases assayed, the isotherms were not different from the control (0% ethanol).

Phase transition points were identified by determining the values of compressibility modulus (K) as a function of molecular areas, from π-area isotherm data using the following equation:

$$K = -(A_\pi)\cdot\left(\frac{\partial \pi}{\partial A}\right)_\pi \quad (2)$$

where A_π is the molecular area at the indicated surface pressure. This parameter reflects the physical state and the bi-dimensional phase transition of the monolayer. The higher the K values, the lower the interfacial elasticity. While in gaseous monolayers, the K value is known to be of the same magnitude as the surface pressure; in liquid phases it varies between 12.5 and 250 mN/m [17]. An abrupt change in K value indicates, with high sensitivity, the onset of a phase transition. Fig. 3b shows the K values corresponding to the isotherms in the upper panel of the same figure (Fig. 3a), in the absence and in the presence of 50 μM FNT in the subphase (other concentrations of FNT are not shown for simplicity). Both conditions evidenced a typical gaseous behavior in K values changing towards a condensed behavior as the molecular area decreased; an abrupt change indicated the bi-dimensional phase transition that, in the presence of FNT, occurred at bigger molecular areas compared to the control without FNT. The condensed nature of the phase state appearing at molecular areas below 50 Å^2 was evidenced by the marked increment in K values and, similarly to what happened with the onset of the phase transition, also occurred before (higher molecular areas) in the presence of FNT than in its absence. This indicated that LC phase of dpPC monolayer was less elastic in presence of FNT [18].

Table 1
FNT per unit area in dpPC monomolecular layers

[FNT] in the subphase (μM)	FNT (pmol) per unit area (mm^2)	
	45 (Å^2)	85 (Å^2)
10	0.558	0.280
20	1.116	0.559
50	2.790	1.398

The molecular areas indicated correspond to approximated surface pressures (π) of 8 and 35 mN/m, respectively. The values were calculated from isotherms shown in Fig. 3a, as explained in Section 2.

The Γ values for FNT, determined from π-area isotherms according to Eq. (1), varied from 0.28 to 0.56 pmol/mm^2 at 10 μM FNT, and from 1.4 to 2.8 pmol/mm^2 at 50 μM FNT in the subphase. These Γ values were calculated at two different pressures, corresponding to two qualitatively different phase states [$\pi = 8$ mN/m (LE) and $\pi = 35$ mN/m (LC)] (Table 1). Plotting Γ as a function of molecular area (Fig. 4), it became evident that the number of FNT molecules per unit area in the monolayer was concentration-dependent but in a non-monotonous manner.

In this last graph, it was possible to distinguish three different behaviors for the values of FNT surface concentration: at the highest molecular areas (the beginning of compression), the Γ values remained constant, then they suffered an important decrease that finished with a marked increment at lower molecular areas. It is interesting to note that the minimum in Γ values (Fig. 4) coincided with both: the minimum in the value of the compressibility modulus (Fig. 3b) and the two-phase (LE–LC) coexisting region of the monolayer (Fig. 3a).

The theoretical amount of FNT per unit area achieved in the monolayer as a function of dpPC molecular area and in the presence of a 50-μM FNT concentration in the subphase, was calculated. We assumed that every FNT molecule placed in the monolayer at the beginning of compression (maximum area) remained in it during the whole compression process, without releasing or incor-

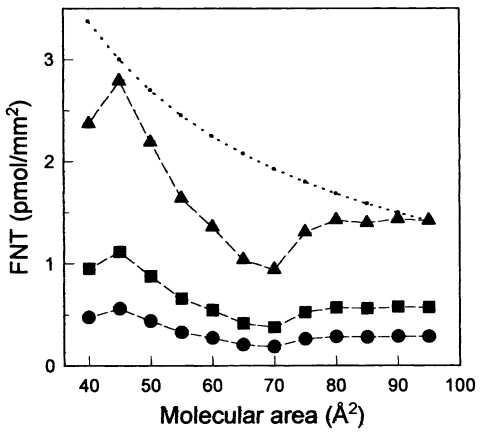

Fig. 4. FNT per unit area present in the dpPC monolayer as a function of dpPC molecular area, at different concentrations of FNT in the subphase: 10 μM (circle), 20 μM (square) and 50 μM (triangle). The values were calculated from isotherms shown in Fig. 3a, according to Eq. (1). The dotted line corresponds to the theoretical amount of FNT per unit area assuming both no changes due to the compression process, in the total amount of FNT present in the monolayer at maximum area, and an ideal mixture between FNT and dpPC.

porating new drug molecules from the subphase (Fig. 4, dotted line). Comparing this theoretical curve with the experimental data, it could be inferred that at high molecular areas (LE phase) the FNT was released from the monolayer while, at low molecular areas (LC phase), it seemed not only to remain but also to increment its concentration in the monolayer by incorporating new drug molecules from the subphase.

Taking these results together, as well as the concept that, in the limits between LE and LC clusters, the system is more disordered that in each separate phase [19], it is possible to suggest that (i) FNT can be more easily incorporated in the monolayer at lower surface pressures as shown in Fig. 2; (ii) once in the monolayer, as the pressure increases, FNT is partially squeezed out until the phase becomes more packed; and (iii) this new molecular arrangement would favor drug binding to the lipidic monolayer in spite of the increment in the surface pressure. Not only the penetration rate of FNT, but also its rate of release are higher in more disordered phases (Figs. 1 and 2), therefore, the increment in drug surface concentration at high π might be thought of as a penetration through expanded phases or boundaries and its subsequent entrapment in the recently-formed condensed phases. Thus, the increase in the Γ values at higher pressures would be explained by either the permanence of FNT molecules trapped in an energetic minimum of binding energy reached at a particular lattice of lipid molecules or the drug physical immobilization inside the growing highly packed LC domains. In both cases, this increment in Γ would be accompanied by the transfer of lipid molecules across the borders between LE and LC phases. Clusters of the LE phase are present through the whole isotherm even above the phase transition region and condensed clusters do not coalesce up to surface pressures higher than the collapse pressure [20]. Moreover, the length of boundary areas increases as the condensed clusters grow [20]; this increment is even more marked for complicated contours such as the curved lobe-shape of dpPC condensed clusters due to the influence of the molecular chirality on the domain shapes [21]. This fact would permit the existence of a net flux of FNT through the LE phase, from the subphase to the LC phase where it is being continuously accumulated. Thus, although FNT hardly penetrates in highly packed monolayers, if penetration mainly occurs when the monolayer is low packed, the drug could remain and it could be concentrated in the lipid layer, even though π increases above the $\pi_{\text{cut-off}}$ value.

From these results (Fig. 4, Table 1) a slower release rate of FNT would have been expected from more condensed layers compared with the less condensed ones. However, the FNT rate of release was rather similar between low and high pressures, as indicated in Fig. 1, probably because the highest rate was reached during the first few minutes when chloroform was still in the monolayer, affecting its properties (see Section 2). FNT concentration in the subphase after 3 h was approximately 1.7 nM. Considering, as found in biomembranes [16], that the FNT-monolayer interaction could be explained by a partition equilibrium model, and taking into account that, in this kind of experiment, FNT molecules were initially incorporated only as a component of the monolayer, we may suggest that the independence from the lateral

pressure of the drug release toward the subphase was due to the fact that the total amount of drug added (incorporated in the interface) was very small compared to the total volume of subphase. Thus, the main force that drove this behavior at low pressures might have been the drug tendency towards the equilibrium concentration between both phases (the monolayer in a LE phase and the subphase).

4. Conclusions

FNT hardly penetrates highly packed monolayers, but it is easily incorporated into low packed ones to an extent sufficient to reach the partition equilibrium. A minimum quantity of drug molecules present in the system, enough to reach a certain threshold value, and a certain level of order in the lipidic phase, are necessary to permit a substantial drug permanence in the membrane.

When FNT molecules present in the monolayers at the LE phase are submitted to increasing lateral surface pressures, the partition equilibrium is displaced toward the aqueous phase and some molecules are expelled from the monolayer. At slightly higher pressures, in the region of phase co-existence, FNT is pushed out even more noticeably, in spite of the increment in monolayer elasticity. In the liquid condensed region, FNT not only remains in the monolayer, but also seems to be incorporated in it, the lipid–FNT interaction and/or FNT entrapment within the lipid lattice being the main force that assures the drug permanence at the monolayer.

The phenomena of FNT penetration in the membrane and release toward the subphase possess different energetic barriers depending on the membrane physical state. In physiological conditions, the penetration and release phenomena would be coupled to local fluctuations of biomembrane packing and in this sense, the present results may contribute to understand the pharmacological effects of BZDs from a more dynamical perspective.

Acknowledgments

This work was supported by grants from FONCYT, SeCyT(UNC), and Agencia Córdoba Ciencia. The authors are career investigators from CONICET (Argentina).

References

[1] J.M. Sloan, E.P. Martin, A. Wala, A comparison of the physical dependence inducing properties of flunitrazepam and diazepam, Pharm. Biochem. Behav. 39 (1991) 395–405.
[2] A.L. Marrow, J.R. Pace, R.H. Purdy, S.M. Paul, Characterization of steroid interactions with gamma-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites, Mol. Pharmacol. 37 (1990) 263–270.
[3] M. Gavish, Hormonal regulation of peripheral-type benzodiazepine receptors, J. Steroid Biochem. Mol. Biol. 53 (1–6) (1995) 57–59.
[4] M.A. Perillo, D.A. Garcia, A. Arce, Partitioning of 1,4-benzodiazepines into natural membranes, Mol. Membr. Biol. 12 (1995) 217–224.
[5] M.A. Perillo, A biophysical approach to the anti-excitotoxic action mechanism of some sphingolipid derivatives, Recent Res. Develop. Lipids Res. 2 (1998) 275–298.
[6] M.A. Perillo, A. Arce, Determination of the membrane-buffer partition coefficient of flunitrazepam, a lipophilic drug, J. Neurosci. Methods 36 (2–3) (1991) 203–208.
[7] A. Seelig, P.R. Allegrini, J. Seelig, Partitioning of local anesthetics into membranes: surface charge effects monitored by the phospholipid head-group, Biochim. Biophys. Acta 939 (2) (1988) 267–276.
[8] D.A. Garcia, M.A. Perillo, Partitioning of flunitrazepam into model membranes studied by temperature controlled gel filtration chromatography, Biomed. Chromatogr. 11 (6) (1997) 343–347.
[9] D.A. Garcia, M.A. Perillo, Benzodiazepine localisation at the lipid–water interface: effect of membrane composition and drug chemical structure, Biochim. Biophys. Acta 1418 (1) (1999) 221–231.
[10] M.A. Perillo, D.A. Garcia, Flunitrazepam induces geometrical changes at the lipid–water interface, Colloids Surf. B 20 (2001) 63–72.
[11] D.A. Garcia, S. Quiroga, M.A. Perillo, Flunitrazepam partitioning into natural membranes increases surface curvature and alters cellular morphology, Chem. Biol. Interact. 129 (3) (2000) 263–277.
[12] D. Marsh, Intrinsic curvature in normal and inverted lipid structures and in membranes, Biophys. J. 70 (1996) 2248–2255.
[13] V.A. Tverdislov, G.B. Khomutov, S.A. Yakovenko, H.D. Youn, A.Y. Bogdanova, I. Bernhardt, Furosemida and DIDS penetration into Langmuir films of stearic acid. The influence of low ionic strength and pH, Colloids Surf. B 5 (1995) 205–211.
[14] M.A. Perillo, A. Polo, A. Guidotti, E. Costa, B. Maggio, Molecular parameters of semisynthetic derivatives of

gangliosides and sphingosine in monolayers at the air–water interface, Chem. Phys. Lipids 65 (1993) 225–238.
[15] G.L. Gaines, Insoluble Monolayers at Liquid–Gas Interfaces, John Wiley & Sons. Inc, New York, 1966.
[16] D.A. Garcia, M.A. Perillo, Supramolecular events modulate flunitrazepam partitioning into natural and model membranes, Colloids Surf. B 9 (1997) 49–57.
[17] D.C. Carrer, B. Maggio, Phase behavior and molecular interactions in mixtures of ceramide with dipalmitoylphosphatidylcholine, J. Lipid Res. 40 (1999) 1978–1989.
[18] J.M. Holopainen, H.L. Brockman, R.E. Brown, P.K. Kinnunen, Interfacial interactions of ceramide with dimyristoylphosphatidylcholine: impact of the *N*-acyl chain, Biophys. J. 80 (2001) 765–775.
[19] M.L. Ruano, K. Nag, L.A. Worthman, C. Casals, J. Perez-Gil, K.M. Keough, Differential partitioning of pulmonary surfactant protein SP-A into regions of monolayers of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylglycerol, Biophys. J. 74 (1998) 1101–1109.
[20] K. Nag, M.W. Keough, Epifluorescence microscopic studies of monolayers containing mixtures of dioleoyl- and dipalmitoylphosphatidylcholines, Biophys. J. 65 (1993) 1019–1026.
[21] P. Kruger, M. Losche, Molecular chirality and domain shapes in lipid monolayers on aqueous surfaces, Phys. Rev. E. 62 (5) (2000) 7031–7043.