Interaction of nicotinamide and picolinamide with phosphatidylcholine and phosphatidylethanolamine membranes: A combined approach using dipole potential measurements and quantum chemical calculations

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ABSTRACT

Interaction between the bioactive compounds nicotinamide and picolinamide and phospholipids (phosphatidylcholines and phosphatidylethanolamines) was investigated by a combined approach using dipole potential measurements and quantum chemical calculations. It is shown that nicotinamide and picolinamide interactions with phosphatidylcholines are of two main types: (i) specific interactions with the phosphate group of the lipid, for which H-bonding between NH_2 group of the substrate and the phosphate plays a dominant role, (ii) conjugated less specific weaker interactions involving both the phosphate and carbonyl groups of the head group, which propagate to the lipid alkyl chains and increase their conformational disorder. For phosphatidylethanolamines, picolinamide was found to decrease the dipole potential of the membrane in a similar way as for phosphatidylcholines, while nicotinamide is ineffective. These findings are correlated with the specific properties of phosphatidylethanolamines (reduced exposure of phosphate groups) and structural differences in the two substrates, in particular: different separation of the nitrogen atoms in the molecules, existence of a strong intramolecular hydrogen bond in picolinamide (NH...N $_{(ring)}$), which is absent in nicotinamide, and non-planarity of nicotinamide molecules, in contrast to picolinamide ones. Additional information on the lipid/substrate interactions was extracted from the analysis of the changes produced in the relevant vibrational frequencies of the lipid and substrate upon binding. The present study gives molecular support to the argument that changes of dipole potentials are due to effects on the constitutive dipolar PO and CO groups. In addition, it is also shown that according to the specific binding of the substrate to one or both of those, the conformational state of the acyl chains may be affected. These entropy effects may be in the origin of the well-known interdependence of the properties of one monolayer with respect to the other in bilayer membranes.

1. Introduction

The elucidation at molecular level of the interactions of bioactive species with phospholipid constituents of biological membranes is of fundamental importance for the description of their mechanisms of action. The ability of bioactive molecules to interact with biomembranes depends in some extent on their possible binding to membrane groups exposed to water, such as carbonyl and phosphate groups of phospholipids, and how they can insert into the hydrophobic region [1,2].

It is well known that lipid membranes are cooperative structures, in the sense that changes at the head groups' region may propagate to the hydrocarbon tails and *vice-versa* [3,4]. However, this visualization of the physicochemical properties of lipid membranes in relation to their response to variations in the external media still faces many unsolved questions. Among them, the interdependence of the properties of one monolayer with respect to the other can be mentioned. This interdependence was put into relevance when the binding constant of Ca^{2+} ions was found to depend on the calcium concentration inside the lipid vesicles [3,5]. That is, the binding of charges on one side of the membrane affects the charges' affinity on the opposite face.

A possible interpretation for this effect is that the conformations of the polar groups of the outer face of the membrane are influenced by those of the polar groups of the inner face. On the other hand, this requires the existence of a coupling between the conformations of the polar head groups of both the inner and outer faces of the bilayer and the conformations of the acyl chains. In particular, in the case of the

binding of Ca^{2+}, the different conformations should result in different exposures of the phosphate groups to the water phase.

In planar black lipid membranes (BLM), it was also shown that electrostriction of charges on one side of the bilayer affects the binding of ions on the other side [6]. Furthermore, it was also shown that this kind of propagation depends on the curvature of the bilayer, this effect being particularly noticeable in small vesicles, in which the packing of the chains in the inner monolayer is considerably more compact than in the outer one [5].

Since the observed synergism between the properties of the two bilayer faces can be related with the distribution of conformations adopted by the phospholipid chains, and hence to the formation of kinks into which water molecules can be clustered, both the conformational properties and water content modulate local changes in the dielectric constant of the bilayer, modifying its properties, like binding affinities and permeability barriers [7].

Coupling between the conformations of the polar head groups of the bilayer and the conformations of the acyl chains is essential but has not yet been fully demonstrated. A way to get an insight on this bilayer property is to study the conformational changes that may occur in the lipid molecules when bioactive species bind to groups that remain exposed to water. These groups are preferentially carbonyls in the glycero-acyl ester union and phosphates in the phospholipids head groups [8].

Among bioactive species, those containing nitrogen atoms are of particular importance due to its direct function or as constituents of nucleic acids. Picolinamide (2-pyridine-carboxamide; PA) and nicotinamide (3-pyridine-carboxamide; NA) (Fig. 1) are two well known bioactive isomers of pyridine-carboxamide [9,10] which were found to take part in many important biological processes—production of energy, synthesis of fatty acids, cholesterol and steroids, signal transduction and maintenance of the integrity of the genome, inhibition of poly(ADP-ribose) synthetase [10–13]—and possess several therapeutic uses (e.g., in diabetes treatment and prevention, osteoarthritis and granuloma annulare [14–17]).

The different relative position of the ring nitrogen atom and carboxamide substituent in NA and PA could be expected to influence the way each molecule binds to the phosphate and carbonyl groups of the membrane phospholipids and, thus, the changes they induce on the membrane surface properties.

A way to determine surface properties is to measure the dipole potential in monolayers spread on the air–water interface at constant area and temperature. In this regard, many studies have shown that the determination in this experimental set up is equivalent to those made directly on bilayers [18,19]. Thus, in the present investigation the effects of the substrates' binding on the surface properties were evaluated by measuring the changes in the dipole potential of monolayers spread on the surface of NA or PA solutions. The interactions of NA and PA with 4 phospholipids derived from phosphatidylcholine and phosphatidylethanolamine were evaluated. In each lipid family, one of the chosen lipids contains both carbonyl and phosphate groups, whereas the second one contains only phosphate groups. The reported effects of interaction between the two studied substrates and the chosen lipids can, in principle, be applied to interactions on both sides of a bilayer.

In the context of the present study, an important difference between phosphatidylcholines and phosphatidylethanolamines is that the last ones show a very strong lateral interaction between the phosphates and the amine groups of adjacent molecules [20–23]. In this direction, FTIR spectroscopy studies have provided information about the strong hydrogen bonding and electrostatic interactions that exist between the head groups in phosphatidylethanolamines [24]. This interaction justifies the smaller volume of the polar head group area in phosphatidylethanolamines with respect to that corresponding to their acyl chains, as well as their low rate of hydration (4–7 water molecules per phosphatidylethanolamine, in comparison to 14–18 water molecules per phosphatidylcholine). In simple words, phosphatidylethanolamines exhibit a reduced trend to interact through their phosphate groups, compared to phosphatidylcholines.

The abovementioned structural differences allowed us to examine the specific role of each type of group (phosphate or carbonyl) in the interaction of the lipids with the chosen substrate molecules. On the other hand, the experimental results obtained from the dipole potential measurements were correlated with conformational changes produced on the phospholipid monolayers upon substrate binding, as evaluated by quantum chemical calculations.

2. Materials

1,2-Dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC), 1,2-di-O-tetradecyl-sn-glycero-3-phosphatidylcholine (PC-ether), 1,2-dimyristoyl-sn-glycero-3-phosphatidylethanol-amine (DMPE) and 1,2-di-O-tetradecyl-sn-glycero-3-phosphatidylethanolamine (PE-ether) were obtained from Avanti Polar Lipids and used as received. Picolinamide (PA) and nicotinamide (NA) were provided by Sigma Chemical (spectroscopic grade) and used without any additional purification.

3. Methods

3.1. Measurement of dipole potentials

Dipole potentials (Ψ_D) were determined in monolayers of the different lipids spread on the air–water interface, an aqueous subphase (KCl, 1 mM) with or without NA or PA. For these measurements, the procedure consists in adding aliquots of a chloroform solution of the lipids on the surface of the aqueous phase until a constant surface pressure was reached (a saturated monolayer of a surface pressure of ca. 44 mN/m) by the evaporation of the solvent [22,24]. A solution of 1 mM of roasted KCL was used in the subphase in all cases. When NA or PA was used, they were dissolved at different concentrations in the mentioned KCl solution [18,19,27].

The values of the interfacial potential (V_{surf}) were determined through a high impedance circuit by means of an Am ionizing electrode (α emissor) placed above the monolayer and a Ag/AgCl reference electrode immersed in the aqueous subphase [18,22,23].

The surface of an aqueous solution, contained in a Teflon trough of fixed area, was exhaustively cleaned. Then, a chloroform solution of the phospholipids was spread on that surface, up to reach a constant surface pressure. The surface pressure of the different lipid monolayers was monitored in a Kibron μ-trough S equipment, at constant temperature and area. The saturation point of the monolayer, for each case, was determined considering the standard deviation of the results at the plateau of the obtained curve. Under these conditions, the measurements of dipole potential were made with lipids in the monolayer in equilibrium with the lipids in the subphase and V_{surf} can be obtained from the following expression:

$$V_{surf} = V_{Ag/AgCl} - V_{grd} = V_{solution} - V_{grd} \qquad (1)$$

where $V_{Ag/AgCl}$ is the potential of the reference electrode and V_{grd} is the potential of the shield covering the ionizing electrode.

Fig. 1. Molecular structures of nicotinamide (NA) and picolinamide (PA).

The dipole potential of the monolayer (Ψ_D) was then evaluated as

$$\Psi_D = V_{surf} - V_{lip} \qquad (2)$$

where V_{surf} is the potential of the clean surface (without lipids) and V_{lip} is the potential after the monolayer was formed.

Different Ψ_D values were obtained as a function of the NA or PA concentrations in the subphase solution. The values of Ψ_D presented are the mean value of at least 5 measurements.

In all experiments, the temperature was measured with a calibrated thermocouple immersed in the subphase and set at the values indicated in each assay (mostly 18 and 28 °C) within ±0.5 °C.

3.2. Theoretical calculations

Theoretical calculations were performed with the GAUSSIAN 03 program [25], at Hartree-Fock level of theory, using the split-valence 3-21G basis set [26]. Structures of the molecules were fully optimized using the program standard methods and convergence criteria [27,28]. Initial structures were guessed by using a simple classical molecular modeling approach (general force field in GaussView [29]) and chemical knowledge about the expected preferred intermolecular interactions. Vibrational frequencies were calculated at the same level of theory and also used to check the nature of the stationary points on the potential energy surface (PES). All structures resulting from the minimization were found to be true minima on the PES, with no negative Hessian eigenvalues. Assignment of the relevant molecular vibrations was performed by direct analysis of the calculated normal modes using GaussView. Binding energies for the lipid//substrate dimers were estimated as the difference between the energy of the dimer and the sum of the energies of the lipid and substrate. Since the focus of the theoretical studies was the evaluation of the specific interactions between the substrates (NA and PA) and the selected lipids, the modeling studies were performed for the molecules or their associates in vacuum and did not take possible environment effects (including presence of water molecules) into account.

4. Results and discussion

As seen in Fig. 2, NA decreases the dipole potential of both DMPC and PC-ether membranes along a concentration range of 500 mM. However, it does not affect significantly the dipole potential of the two phosphatidylethanolamine lipids (DMPE and PE-ether). These results indicate that the presence of phosphate groups non-bounded to adjacent amine fragments is required for NA insertion into the membrane. In contrast, within the same concentration range, PA decreases in a similar way the dipole potential of the membranes of all studied lipids (Fig. 3).

In order to shed light on the relative strength and possible cooperative effects in the membrane of the interaction between the selected substrates and the phosphate and carbonyl groups of the lipids, which are those determining the dipole potential [21,22], simple quantum chemical calculations on isolated dimers of the DMPC and PC-ether with NA and PA were performed. Table 1 gives the energies of the optimized structures of isolated DMPC, PC-ether, NA, PA and dimers between NA and PA with DMPC and PC-ether. In the case of DMPC, both interactions with phosphate and carbonyl groups were considered.

The main conclusions regarding energies are the following: (i) NA interacts more strongly with both DMPC and PC-ether than PA; this result correlates well with the fact that ca. 70 % of the saturation value of the dipole potential is obtained at much lower values of concentration in NA than PA (10 mM for NA in comparison to 100 mM for PA) (see Figs. 2 and 3). (ii) For both NA and PA, the interaction with the phosphate group of DMPC is stronger than that with the carbonyl groups; this may account for the observed general
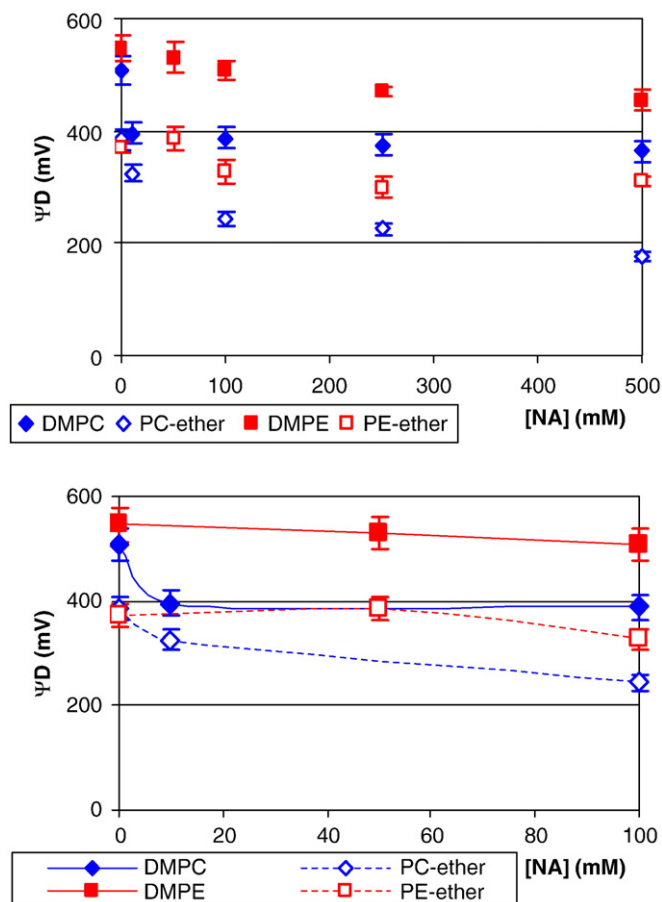
trend found for the studied substrates (specially NA) to interact with the membranes mainly through the phosphate groups of the lipids, as deduced from the dipole potential measurements, where the decrease in dipole potential for ester and ether PC is similar. (iii) Also for both NA and PA, the interaction with DMPC via phosphate group is only slightly stronger than that with PC-ether, whereas the substrates' interaction with DMPC via carbonyl groups is slightly weaker than this latter. Note that the experimentally observed dipole potential changes upon binding of the substrates are found to be approximately equal in
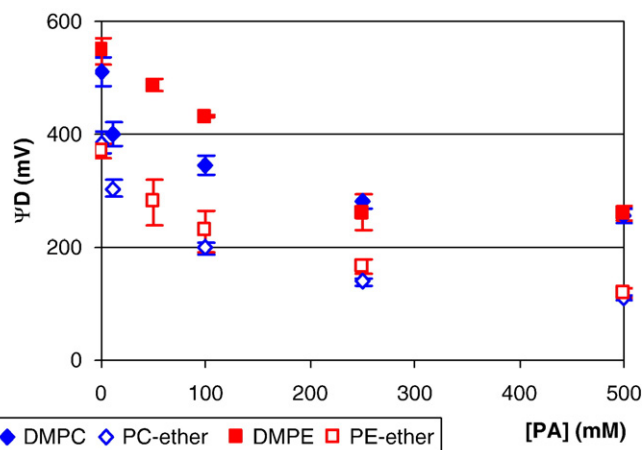

Fig. 2. Effect of nicotinamide (NA) on the dipole potential (Ψ_D) of DMPC, PC-ether, DMPE and PE-ether monolayers.

Fig. 3. Effect of picolinamide (PA) on the dipole potential (Ψ_D) of DMPC, PC-ether, DMPE and PE-ether monolayers.

Table 1
Calculated binding energies (ΔE_b/kJ mol^{-1}) for the isolated lipid//substrate (lipid: DMPC; PC-ether; substrate: NA or PA) dimers.[a]

| Lipid//Substrate	Interacting groups[b]	E	ΔE_b	$\Delta[\Delta E_b]$ (phosphate-carbonyl)
DMPC//NA	P	−7,347,215.60	−107.44	−10.57
DMPC//NA	C	−7,347,205.03	−96.87	
DMPC//PA	P	−7,347,227.09	−86.23	−25.82
DMPC//PA	C	−7,347,201.28	−60.41	
PC-ether//NA	P	−6,962,226.01	−102.00	
PC-ether//PA	P	−6,962,239.85	−83.14	

[a] Calculated total energies (*E*, obtained at the HF/3-21G level of theory) for DMPC, PC-ether, NA and PA are −6,264,983.13, −5,879,998.98, −1,082,125.02 and −1,082,157.73 kJ mol^{-1}, respectively.
[b] P, phosphate groups; C, carbonyl groups.

the two lipids (see Figs. 2 and 3), what may then be interpreted as resulting from the conjugated opposite effect of the two types of interaction between the substrates and DMPC (via phosphate and via carbonyl groups).

If we look now to the optimized structures of the various DMPC-based lipid//substrate dimers (Fig. 4), we can extract the following main conclusions:

(i) For both NA and PA interacting with DMPC, the calculations revealed that when the interaction is with the phosphate group, the alkyl chains of the lipid do not change appreciably of conformation, staying nearly parallel to each other as in the most stable structure of isolated DMPC, whereas the weaker interaction with the carbonyl groups induces important conformational changes in the vicinity of the head group of the lipid, making the alkyl chains to separate considerably from each other. If a similar phenomenon occurs when the substrates are interacting with a DMPC-based membrane, this shall lead to increased entropy of the lipid alkyl chains, which may then favor substrate insertion in the membrane. At the same time, this type of conformational changes induced in the alkyl chains may also be in the origin of the known interdependence of the properties of one monolayer with respect to the other in bilayer membranes [5,30].
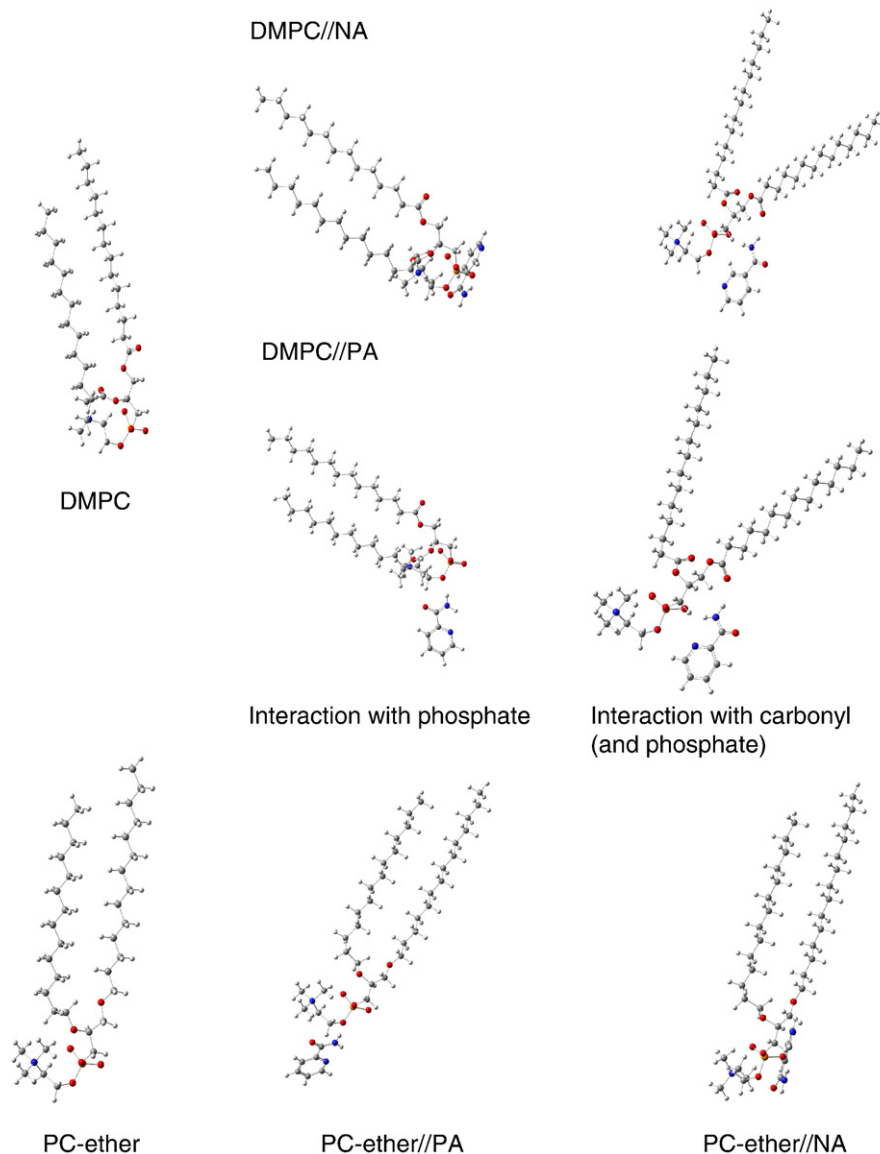
Fig. 4. Conformational changes in the alkyl chains induced by substrate (PA or NA) binding to DNPC and PC-ether as predicted by the HF/3-21G calculations. In the first case, interactions with both phosphate and carbonyl groups are considered. The drawings of the optimized structures are shown keeping the phosphate group with the same orientation.

(ii) Interestingly, when NA or PA interacts with the carbonyls of DMPC, they also interact in some extent with the phosphate. We can say that it is exactly because of this (to allow for maximum energy stabilization through interaction with both groups) that the DMPC molecule folds around the head group and obliges the two chains to separate from each other. This result is also in agreement with the observed general trend found for the studied substrates (in particular NA) to interact with the membranes mainly through the phosphate groups of the lipids, as deduced from the dipole potential measurements.

(iii) Interactions of NA and PA with PC-ether appear to be similar to those with the phosphate group of DMPC also from the structural point of view.

(iv) NA molecules seem to stabilize rather parallel to the membrane plane while PA does it normally to the plane. As the dipole potential is the vector sum of different dipole components normal to the interphase, the disposition adopted by PA promotes a higher decrease in the monolayer dipole potential than NA.

In summary, the theoretical studies on the DMPC-based lipids interactions with NA and PA, indicate that these interactions may be of two main types: (i) specific interactions between the substrate with the phosphate group of the lipid, for which with all probability H-bonding between NH_2 group of the substrate and the phosphate plays a dominant role, and (ii) conjugated less specific weaker interactions involving the head group (where both the phosphate and carbonyl groups may be involved) propagate to lipid alkyl chains increasing their conformational disorder.

Let us now consider the case of the interactions between NA and PA with the studied phosphatidylethanolamine lipids, PE and PE-ether. As already mentioned, the main difference between NA and PA is the fact that within the same concentration range PA decreases in a similar way the dipole potential of the membranes of all studied lipids, while NA is practically ineffective in the case of the two phosphatidylethanolamine lipids (see Figs. 2 and 3). This must be a consequence of at least one the three main characteristics distinguishing the two substrates: different separation of the nitrogen atoms in the molecules, which is larger for NA than for PA; existence of a strong intramolecular hydrogen bond in PA (NH...N $_{(ring)}$), which is absent in NA; and non-planarity of NA molecules, in contrast to PA ones which are planar [31,32].

Since in the phosphatidylethanolamine lipids the phosphate groups are known to be tightly bound to adjacent PE's via amine group, limiting its hydration [24], PA must necessarily interact with membranes of these lipids through a mechanism where specific direct interactions with the phosphate groups are not dominant, i.e., through a mechanism of the second type described above, involving less specific interactions with the head group and effective introduction of conformational disorder in the alkyl chains. Such kind of mechanism seems in fact to be consistent with a greater difficulty of the substrate to interact with the phosphate groups through hydrogen bonding, that is exactly what we can expect when comparing PA with NA, since in the former molecule there is a strong intramolecular H-bonding interaction (absent in NA). It is then possible that, when the PA molecules cannot bind efficiently to the phosphate groups, as in the presence of phosphatidylethanolamine-based membranes where these latter groups are not easily accessible, a substantial fraction of PA molecules can keep their intramolecular hydrogen bond and penetrate the membrane, thus inducing conformational disorder. This conformational disorder can then promote a membrane expansion and, consequently, a decrease in the dipole potential. The insertion of PA in the membrane may also be facilitated by the fact that its molecules are planar (due to geometrical restrictions imposed by the intramolecular H-bond), whereas those of NA are not [31,32]. This can

also be visualized considering that the intramolecular H-bond of PA makes this molecule less hydrophilic and hence more willing to partition into the alkyl chains of the membrane.

The above proposed mechanisms, essentially specific via phosphate groups for DMPC-based lipids, and less specific but leading to effective introduction of conformational disorder in the alkyl chains for PA interaction with phosphatidylethanolamine-based lipids, fully explain the experimental observations regarding changes in the dipole potentials for the different pairs of lipid//substrate and are also in agreement with the theoretical results for isolated dimers of DMPC-based lipids with NA and PA.

Further information could be extracted from the comparison of calculated infrared spectra of DMPC and PC-ether in the presence and absence of NA and PA (Fig. 5) with relevant experimental infrared spectra assignments available for phospholipids (Table 2). Note that the calculated wavenumbers presented in both Fig. 5 and Table 2 were not scaled to correct them from anharmonicity effects and limitations of the theoretical approaches used (e.g., absence of consideration of electron correlation and limited size of the basis set) and refer to isolated molecules or associates in vacuum, so that absolute values are expected to generally overestimate the experimental ones, except in cases where cancellation of errors takes place. On the other hand, relative wavenumbers' values are expected to be meaningful.

We analyzed both the carbonyl (1850–1600 cm^{-1}) and phosphate (1250–950 cm^{-1}) stretching regions. In the last region, the γNH_2 (out of plane rocking) mode of the NA or PA and the rocking modes of the DMPC carbonyl groups also absorb strongly, complicating in some extent the analysis. In this region, the vibrations are also considerably mixed, in particular the NH_2 and PO_2 symmetric stretching modes.

Very interestingly, when NA and PA bind DMPC or PC-ether, their δNH_2 and $\nu C{=}O$ modes couple extensively and among the two bands originated in these coordinates, the predominant contribution in the higher frequency band is due to δNH_2 (which for the isolated NA and PA molecules absorbs at a lower frequency), whereas the main contribution to the lower frequency band is due to the $C{=}O$ mode (see Fig. 5a and b). The composition of the relevant modes could be easily done by direct analysis of the calculated normal modes using GaussView normal modes animation module. The results for the PC-ether are easier to interpret because this molecule does not have carbonyl groups. So, in the carbonyl stretching region, we can notice clearly how the δNH_2 and $\nu C{=}O$ modes of NA and PA change their frequencies upon binding. As it could be expected, δNH_2 increases its frequency due to H-bonding (it becomes more difficult to bend the amine group which in the complex has the hydrogen atoms taking part in a H-bond) and $\nu C{=}O$ decreases because there is some electron release from the carbonyl bond to the $C{-}NH_2$ fragment from where the H-bond with PC-ether withdraws electrons. These trends are also observed for NA and PA interactions with DMPC.

In the phosphate region of the PC-ether (Fig. 5f), the νPO_2 asymmetric stretching reduces upon complexation with PA and more with NA, which indicates a stronger interaction of the phosphate group with NA than with PA, in agreement with both the dipole potential measurements as well as with the theoretical structural and binding energies data obtained for the dimeric structures, already discussed. The analysis of the νPO_2 symmetric stretching is more complex due to superposition with the intense bands due to γNH_2 (which in the isolated substrates absorbs at much lower frequencies, as given in the figure; see below). Apparently, there are two bands with important contribution from the νPO_2 symmetric stretching, one at higher frequency than for isolated PC-ether and the other one at lower frequency.

The analysis of the changes in the γNH_2 mode is not straightforward because in isolated NA there is only a vibration, whereas in PA the description of the γNH_2 rocking modes is better done if we consider one out of plane mode for the intramolecularly bound hydrogen and another one for the "free" hydrogen. The frequencies of these two modes in isolated PA are quite different (662 *vs.* 601 cm^{-1}) and this
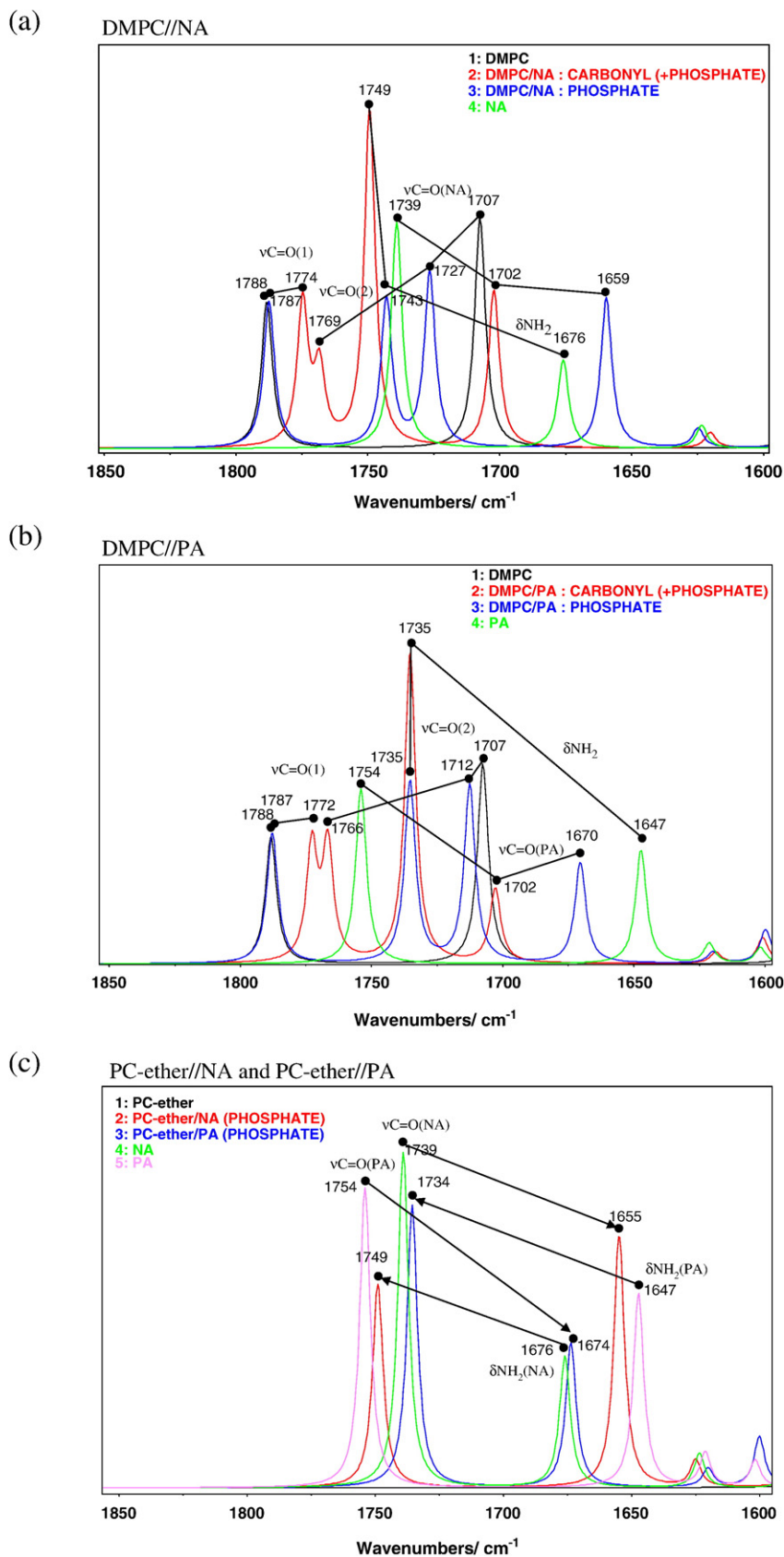
Fig. 5. Simulated infrared spectra of isolated DMPC, PC-ether, NA and PA and dimers (lipid//substrate) of these species as predicted at the HF/3-21G level of theory. The spectra were simulated using Gaussian functions centered at the calculated wavenumbers with constant width at half height (20 cm^{-1}); areas are proportional to the calculated band intensities. Panels a–c refer to the carbonyl (1850–1600 cm^{-1}) stretching region and panels d–f to the phosphate (1250–950 cm^{-1}) stretching region.
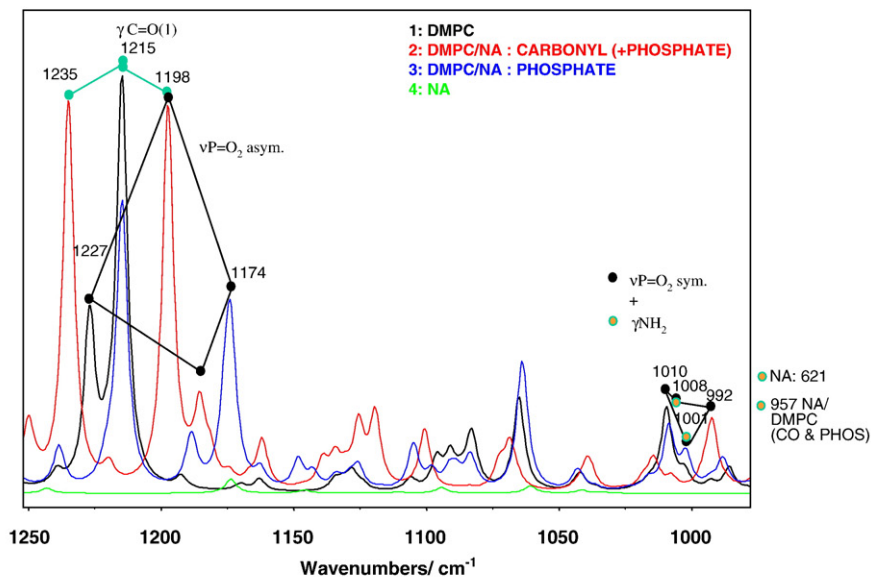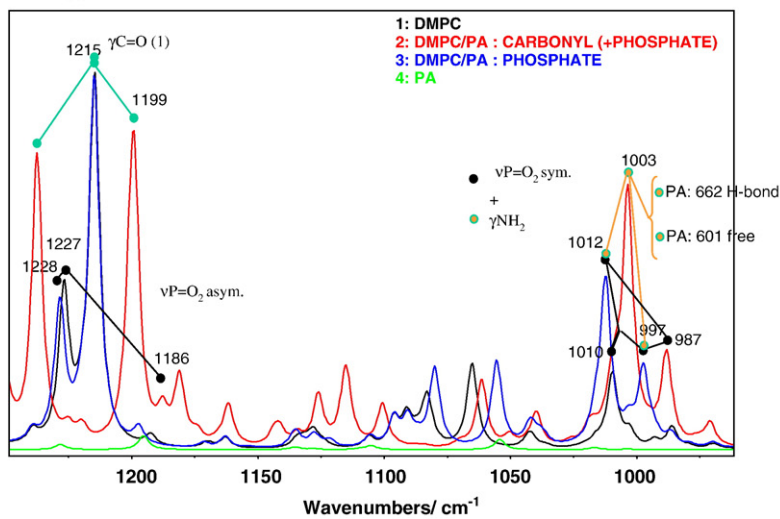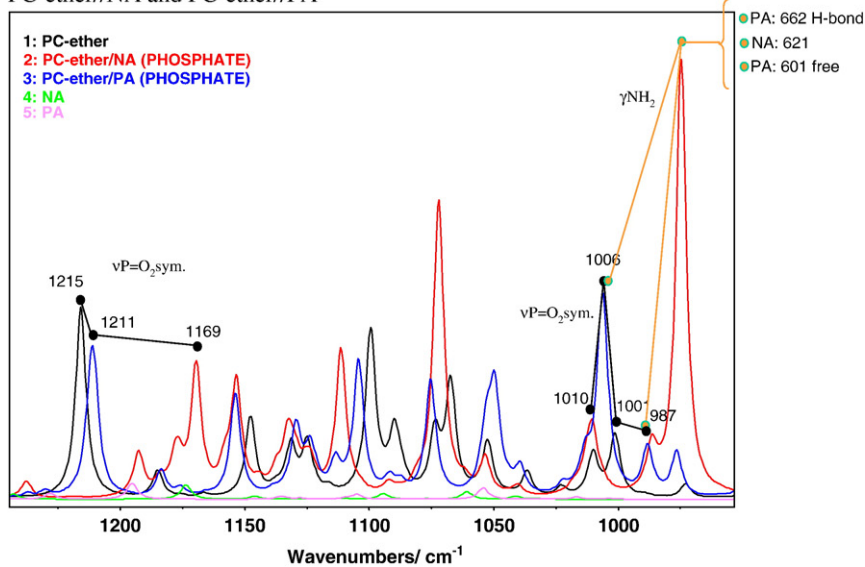
Fig. 5 (*continued*).

Table 2
Selected HF/3-21G calculated vibrational wavenumbers (cm^{-1}) of NA, PA, phosphate and carbonyl groups of DMPC and PC-ether and of dimers of these lipids with NA and PA.

Vibration	NA	PA	DMPC	DMPC//NA		DMPC//PA		PC-ether	PC-ether//NA	PC-ether//PA
				P[a]	C(+P)[b]	P[a]	C(+P)[b]			
νC=O (lipid)			(1) 1788	1788	1774	1787	1772			
			(2) 1707 (1710)[c]	1727	1769	1712	1766			
νC=O (substrate)	1739 (1677)[d]	1754 (1686)[d]		1659	1702	1670	1702		1655	1674
δNH$_2$	1676 (1627)[d]	1647 (1571)[d]		1743	1749	1735	1735		1749	1734
νPO$_2$ asym			1227 (1230)[c]	1174	1180	1228	1186	1215	1169	1211
νPO$_2$ sym			1010 (1086)[c]	1008	1012	1012	1012	1001	1010	1006
				1001	992	997	987		987	987

Reference experimental data are in parentheses.
[a] Interaction via phosphate group of the lipid.
[b] Interaction via carbonyl (+ phosphate) group of the lipid.
[c] Experimental data obtained for dispersed DMPC in a full hydrated state [33]. Contributions from both carbonyl groups was observed, only as one broad band centered at the given frequency, (1), carbonyl group interacting directly with the substrate; (2), carbonyl group indirectly affected by the interaction (see text).
[d] In the neat glassy state of the compound at 10 K [32].

makes the comparison of the shifts in the NH$_2$ rocking modes upon binding of the two molecules (NA and PA) to PC-ether not conclusive.

If we consider now the results for DMPC (Fig. 5d and e), interaction with the phosphate group leads essentially to the same predicted spectral changes as in PC-ether. This is true for both interaction with NA and PA.

The changes in the frequencies of the carbonyl groups of DMPC (Fig. 5a and b) follow the following trends: (i) the directly interacting carbonyl group frequency changes to lower values; when the interaction is with the phosphate group, the frequency of this carbonyl group does not change relatively to the isolated DMPC molecule; (ii) the non-directly interacting carbonyl group frequency increases slightly from isolated DMPC to DMPC interacting via phosphate group, and increases considerably when interaction is via carbonyl; this shows the fact that the carbonyl not directly involved in the interaction with NA and PA experiences a strong change in its chemical environment, as it in fact happens due to the observed separation of the two lipid alkyl chains in this case; apparently, this leads to a strengthening of that carbonyl bond; (iii) both effects described in (i) and (ii) are more relevant for NA than PA, once again expressing the idea that NA interacts strongly with DMPC than PA (as indicated in all the remaining theoretical and experimental data obtained in this study and already discussed above).

As already mentioned, the main objective of the analysis of the effects of substrates' binding to the studied lipids on the theoretically predicted IR spectra was to obtain further support to the conclusions extracted from the theoretical structural analysis and from dipole measurements, in particular to obtain further details about the specific interactions considered in our study. In that sense, the theoretical results are by far more powerful than any experimental data obtained by measuring the IR spectra of the systems under study by the following main reasons. (i) Experimentally, we do not have access to the characterization of the vibrations. So, for example, we could not easily find that the δNH$_2$ and νC=O modes of NA and PA change their frequency order upon binding due to specific H-bond interactions with the lipids. To have the chance to eventually check this point, study of isotopically labeled substrates could in principle be suggested. However, rapid H/D-exchange with solvent would preclude to extract any conclusion from using deuterated samples, and even the expensive (and complex from the point of view of synthetic work) experiments involving oxygen labeled isotopologues of NA and PA with all probability would not also allow for any conclusion regarding this point, since the expected wavenumbers' shifts upon ^{16}O to ^{18}O replacement are minimal and the bandwidths of the experimental bands are large. (ii) In the interactions involving DMPC, experimental measurements would not give any information about the different effects resulting from interaction with the phospholipid carbonyl or phosphate groups, since both types of interaction take place simultaneously. Then, the only relevant information that could be devised

to be possible to obtain from analysis of the experimental data relates with the interactions between the substrates (NA and PA) and the phosphate group of the PC-ether, which has no carbonyl group. However, as already mentioned, the νPO$_2$ symmetric stretching region of the IR spectrum is, even in the theoretical spectra, quite complex, due to occurrence of other intense modes in the same spectral range (e.g., γNH$_2$). In addition, it must be also taken into account that the experimental bands have large widths and then extensive band overlapping takes place, making the analysis of the experimental data even more difficult and uncertain. Nevertheless, we recorded the infrared spectra of dried PC-ether and both PC-ether//NA and PC-ether//PA systems at room temperature in KBr pellet and, in spite of all the above mentioned intrinsic limitations of the experiments, the analysis of both the νPO$_2$ asymmetric stretching and vCO/δNH$_2$ spectral regions (Figs. 6 and 7) reveals a good agreement between the experimental data and the theoretical predictions. Hence, in the first spectral region (Fig. 6), the experimental band in the neat phospholipid was observed at 1243 cm^{-1}, reducing upon complexation with PA to ca. 1233 cm^{-1} and consider-
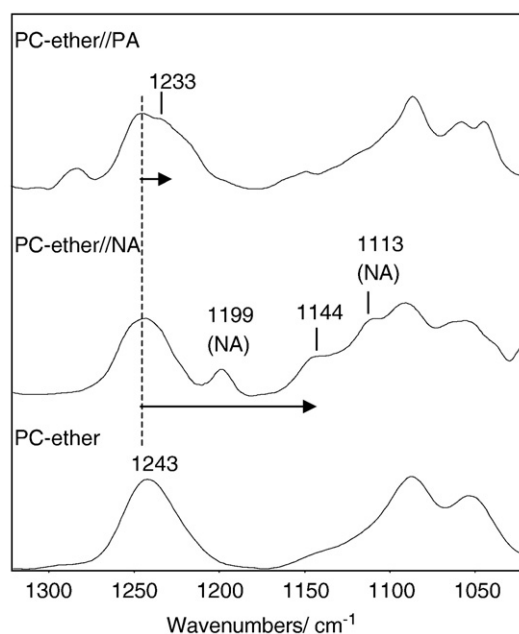
Fig. 6. Infrared spectra (νSO$_2$ asym. Stretching region) of dehydrated PC-ether, PC-ether//NA and PC-ether//PA systems in KBr Pellet. The bands at 1113 and 1199 cm^{-1} are bands due to NA and are found at nearly the same values in the spectra of neat NA in KBr pellet. The arrows indicate the observed shifts due to interaction between the substrates (NA and PA) and the phospholipid. The spectra were obtained in a BOMEM MB104 FT-IR spectrometer with 4 cm^{-1} resolution and 128 co-added scans.
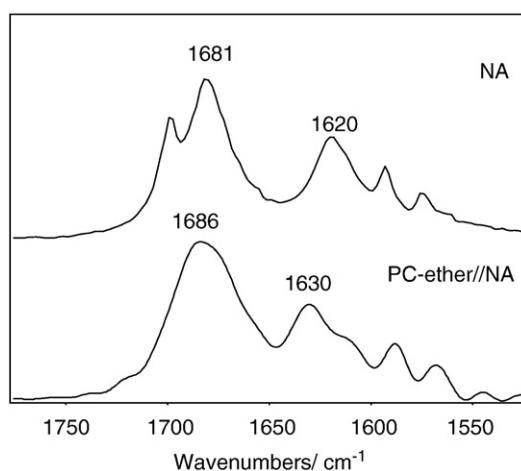
Fig. 7. Infrared spectra (substrate νCO and δNH$_2$ region) of dehydrated PC-ether//NA and neat NA in KBr Pellet. The spectra were obtained in a BOMEM MB104 FT-IR spectrometer with 4 cm^{-1} resolution and 128 co-added scans.

ably more upon complexation with NA (1144 cm^{-1}). In the second spectral region (Fig. 7), the main bands of NA originated in the νC=O and δNH$_2$ modes were observed at 1681 and 1620 cm^{-1}, respectively, and, in consonance with the theoretical predictions (see Fig. 5c), shift to 1630 and 1686 cm^{-1}.

5. Conclusions

From dipole potential measurements and quantum chemical calculations, the mechanisms of interaction of NA and PA with phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) were evaluated. It was shown that the interactions of both NA and PA with PCs are of two main types: (i) specific interactions with the phosphate group of the lipid, mainly through H-bonding involving the amide group of the substrate, and (ii) less specific weaker interactions involving both the phosphate and carbonyl groups of the lipid head groups, which propagate to the lipid alkyl chains and increase their conformational disorder. These entropy effects may be the origin of the well-known interdependence of the properties of one monolayer with respect to the other in bilayer membranes.

For PEs, PA was found to decrease the dipole potential of the membrane in a similar way as for PCs, while NA is ineffective. These findings could be correlated with structural differences in the two substrates, in particular: different separation of the nitrogen atoms in the molecules, existence of a strong intramolecular hydrogen bond in PA (NH...N (ring)), which is absent in NA, and non-planarity of NA molecules, in contrast to PA ones. Since in the phosphatidylethanolamine lipids the phosphate groups are known to be tightly bounded to adjacent NH$_2$ groups [24], PA must interact with membranes of these lipids through a mechanism where specific direct interactions with the phosphate groups are not dominant, i.e., a mechanism of the second type above described, involving less specific interactions with the head group and leading to an effective introduction of conformational disorder in the alkyl chains. This mechanism is clearly favored for PA, compared to NA, due to the strong intramolecular H-bond existing in the former molecule. In the presence of phosphatidylethanolamine-based membranes, a substantial fraction of PA molecules can keep their intramolecular hydrogen bond and penetrate the membrane, thus inducing conformational disorder and changing the membrane dipole potential.

Additional information on the lipid/substrate interactions was extracted from the analysis of the changes produced in the relevant vibrational frequencies of the lipid and substrate upon binding, which fully support the conclusions extracted from the analysis of the dipole potential and structural data.

References

[1] W. Caetano, M. Ferreira, M. Tabak, M.I.M. Sanchez, O.N. Oliveira, P. Kruger, M. Schalke, M. Losche, Cooperativity of phospholipid reorganization upon interaction of dipyridamole with surface monolayers on water, Biophys. Chem. 91 (1) (2001) 21–35.

[2] S.H. White, Membrane protein insertion: the biology-physics nexus, J. Gen. Physiol. 129 (5) (2007) 363–369.

[3] E.A. Disalvo, Divalent-cations promote transversal perturbations in lipid bilayers of sonicated vesicles, Bioelectrochem. Bioenerg. 11 (2–3) (1983) 145–154.

[4] B. Bechinger, J. Seelig, Interaction of electric dipoles with phospholipid head groups—a H^2 and P^{31} NMR-study of phloretin and phloretin analogs in phosphatidylcholine membranes, Biochemistry 30 (16) (1991) 3923–3929.

[5] E.A. Disalvo, L.S. Bakas, Stoichiometry of the binding of Ca^{2+} on the inner and the outer monolayers of sonicated vesicle bilayers, Bioelectrochem. Bioenerg. 20 (1–3) (1988) 257–267.

[6] S. Ohki, Effects of divalent-cations, temperature, osmotic-pressure gradient, and vesicle curvature on phosphatidylserine vesicle fusion, J. Membr. Biol. 77 (3) (1984) 265–275.

[7] L.S. Bakas, E.A. Disalvo, Effect of the asymmetric Ca^{2+} distribution on the bilayer properties of phosphatidylcholine-sonicated vesicles, Biochim. Biophys. Acta 979 (3) (1989) 352–360.

[8] E.A. Disalvo, F. Lairion, F. Martini, E. Tymczyszyn, M. Frias, H. Almaleck, G.J. Gordillo, Structural and functional properties of hydration and confined water in membrane interfaces, Biochim. Biophys. Acta 1778 (12) (2008) 2655–2670.

[9] T. Kawabata, T. Ogino, M. Mori, M. Awai, Effects of nicotinamide and its isomers on iron-induced renal damage, Acta Pathol. Jpn. 42 (7) (1992) 469–475.

[10] L.K. Klaidman, S.K. Mukherjee, T.P. Hutchin, J.D. Adams, Nicotinamide as a precursor for NAD(+) prevents apoptosis in the mouse brain induced by tertiary-butylhydroperoxide, Neurosci. Lett. 206 (1) (1996) 5–8.

[11] E.L. Jacobson, W.M. Shieh, A.C. Huang, Mapping the role of NAD metabolism in prevention and treatment of carcinogenesis, Mol.Cell. Biochem. 193 (1–2) (1999) 69–74.

[12] J.M. Denu, Vitamin B-3 and sirtuin function, Trends Biochem. Sci. 30 (9) (2005) 479–483.

[13] H. Yamamoto, H. Okamoto, Protection by picolinamide—a novel inhibitor of poly (adp-ribose) synthetase, against both streptozotocin-induced depression of proinsulin synthesis and reduction of NAD content in pancreatic-islets, Biochem. Biophys. Res. Commun. 95 (1) (1980) 474–481.

[14] R.B. Elliott, C.C. Pilcher, A. Stewart, D. Fergusson, M.A. McGregor, The use of nicotinamide in the prevention of type-1 diabetes, in: A.C. Allison, K.J. Lafferty, H. Fliri (Eds.), Immunosuppressive and Antiinflammatory Drugs, Vol. 696, 1993, pp. 333–341.

[15] M.T. Behme, Nicotinamide and diabetes prevention, Nutr. Rev. 53 (5) (1995) 137–139.

[16] J.P. Kamat, T.P.A. Devasagayam, Nicotinamide (vitamin B-3) as an effective antioxidant against oxidative damage in rat brain mitochondria, Redox Rep. 4 (4) (1999) 179–184.

[17] R.W. Pero, B. Axelsson, D. Siemann, D. Chaplin, G. Dougherty, Newly discovered anti-inflammatory properties of the benzamides and nicotinamides, Mol. Cell. Biochem. 193 (1–2) (1999) 119–125.

[18] R.C. Macdonald, S.A. Simon, Lipid monolayer states and their relationships to bilayers, Proc. Natl. Acad. Sci. U. S. A. 84 (12) (1987) 4089–4093.

[19] H. Brockman, Lipid monolayers: why use half a membrane to characterize protein-membrane interactions? Curr. Opin. Struct. Biol. 9 (4) (1999) 438–443.

[20] K. Gawrisch, D. Ruston, J. Zimmerberg, V.A. Parsegian, R.P. Rand, N. Fuller, Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces, Biophys. J. 61 (5) (1992) 1213–1223.

[21] S. Diaz, F. Amalfa, A.C.B. de Lopez, E.A. Disalvo, Effect of water polarized at the carbonyl groups of phosphatidylcholines on the dipole potential of lipid bilayers, Langmuir 15 (15) (1999) 5179–5182.

[22] S. Diaz, F. Lairion, J. Arroyo, A.C.B. de Lopez, E.A. Disalvo, Contribution of phosphate groups to the dipole potential of dimyristoylphosphatidylcholine membranes, Langmuir 17 (3) (2001) 852–855.

[23] R.J. Clarke, The dipole potential of phospholipid membranes and methods for its detection, Adv. Colloid Interface Sci. 89 (2001) 263–281.

[24] A.M. Bouchet, M.A. Frias, E. Lairion, F. Martini, H. Almaleck, G. Gordillo, E.A. Disalvo, Structural and dynamical surface properties of phosphatidylethanolamine containing membranes, Biochim. Biophys. Acta-Biomembr. 2009 (5) (1788) 918–925.

[25] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.J.A. Montgomery, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K.

Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, GAUSSIAN 03 (Revision B. 01), Gaussian, Inc., Wallingford CT, 2004.

[26] J.S. Binkley, J.A. Pople, W.J. Hehre, Self-consistent molecular-orbital methods. 21. Small split-valence basis-sets for 1st-row elements, J. Am. Chem. Soc. 102 (3) (1980) 939–947.

[27] C.Y. Peng, P.Y. Ayala, H.B. Schlegel, M.J. Frisch, Using redundant internal coordinates to optimize equilibrium geometries and transition states, J. Comput. Chem. 17 (1) (1996) 49–56.

[28] P. Csaszar, P. Pulay, Geometry optimization by direct inversion in the iterative subspace, J. Mol. Struct. 114 (1984) 31–34.

[29] R. Dennington II, T. Keith, J. Millam, GaussView, Version 4.1, Semichem, Inc., Shawnee Mission, KS, 2007.

[30] J.Y.A. Lehtonen, P.K.J. Kinnunen, Changes in the lipid dynamics of liposomal membranes induced by poly(ethylene glycol)—free-volume alterations revealed by intermolecular and intramolecular excimer-forming phospholipid analogs, Biophys. J. 66 (6) (1994) 1981–1990.

[31] A. Borba, A. Gomez-Zavaglia, R. Fausto, Molecular structure, vibrational spectra, quantum chemical calculations and photochemistry of picolinamide and isonicotinamide isolated in cryogenic inert matrixes and in the neat low-temperature solid phases, J. Phys. Chem. A 112 (1) (2008) 45–57.

[32] A. Borba, M. Albrecht, A. Gomez-Zavaglia, L. Lapinski, M.J. Nowak, M.A. Suhm, R. Fausto, Dimer formation in nicotinamide and picolinamide in the gas and condensed phases probed by infrared spectroscopy, Phys. Chem. Chem. Phys. 10 (46) (2008) 7010–7021.

[33] M.A. Frias, S.B. Diaz, N.M. Ale, A. Ben Altabef, E.A. Disalvo, FTIR analysis of the interaction of arbutin with dimyristoyl phosphatidylcholine in anhydrous and hydrated states, Biochim. Biophys. Acta-Biomembr. 1758 (11) (2006) 1823–1829.