

Phospholipid adsorption from vegetable oils on acid-activated sepiolite

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Received: 24 March 2015 / Revised: 4 June 2015 / Accepted: 23 June 2015 / Published online: 29 June 2015
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Abstract In this study, adsorption of natural and model phospholipids (PL) from camelina and rapeseed oils on acid-activated sepiolite has been studied. Adsorption isotherms were determined for total phosphorus and for individual PL at different conditions. Influence of acid-degumming with citric acid as well as of type and ionic form of the phospholipid was investigated. The experimental data were correlated using the Moreau isotherm. The results show that neutral PLs, such as phosphatidylcholines, adsorb as ordered monolayers with molecular area of 1.3 nm²/molecule. Phosphatidic acids have similar adsorption capacity when in free acid form but the interaction with the clay surface is much weaker. Sodium salt gives substantially lower saturation capacity because of electrostatic repulsion between the adsorbed PL molecules and between PL and the clay surface. Calcium and magnesium salts have very low capacity and seem to be incapable to form any ordered structures on the clay surface.

Keywords Acid-activated sepiolite · Phospholipids · Vegetable oil · Adsorption

1 Introduction

Phospholipids (PL) are biologically essential compounds that contain hydrophilic phosphate group and one or two hydrophobic fatty acid residues linked by glycerol. Large number of PL's is known including e.g. phosphatidic acids (PA), phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidyl-inositols (PI). Depending on the length and degree of unsaturation of the fatty acids as well as the substituents at the phosphate group, a wide spectrum of properties can be found. PL's have been classified according to solubility or swelling in water and Pichot et al. (2013) have used four classes I, II, IIIA and IIIB. Class I includes long-chain PL's with very little water swelling and they are commonly referred to as non-hydratable PL. Waxes and some metal salts of long-chain PA belong to this category. Class II PL's still have very low solubility in water but they swell and are thus considered as hydratable PL. Long-chain PC and PE are typical representatives of this class (Hancer et al. 2002; Deffense 2009). Water-soluble PLs with short hydrocarbon chains are included in classes IIIA and IIIB.

Due to the chemical composition, PL are amphiphilic and tend to form ordered mono- or bilayers at interfaces. In aqueous systems, bilayers are more favored while monolayers are preferred in oils (Pichot et al. 2013). It is well known that PL bilayers are the main building blocks of biological membranes and therefore PL are always present in raw materials of biological origin. PL impair stability against oxidation and are harmful in vegetable oil hydrogenation (Hitchman 2009) and in bio-fuel production (Vera et al. 2011). Purification or degumming methods are thus essential in refining biological materials, such as vegetable oils. Typically, phosphorus contents less than 10 mg/kg are needed for industrial applications (Vera et al. 2011).

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Moreover, metal cations like Na^+ , Ca^{2+} , Mg^{2+} and Fe^{2+} are frequently bound at the phosphate end group of PA thus changing the properties of the parent PA.

Large number of purification processes has been proposed depending on the amount and properties of the PL present in the feedstock (Vera et al. 2011; van Nieuwenhuyzen and Tomás 2008). Adsorption has been conventionally used to remove various impurities, including PL, from oils (Yu et al. 2013). Silica and different clay minerals are widely utilized due to their good uptake capacity, surface properties, cost and ease of operation (Vera et al. 2011; Rossi et al. 2003). The clays used in oil purification include, for example, natural and acid-activated sepiolite (Sabah and Majdan 2009; Sabah et al. 2007), palygorskite (Galan 1996) and attapulgite (Liu et al. 2008).

In spite of the large-scale utilization of clay adsorption, mechanisms involved in phospholipid attachment on the solid surface are far from clear. Large number of studies has been devoted on behavior of PL at air/water and oil/water interfaces, and they were recently reviewed by Pichot et al. (2013). Much less information is available on the behavior at solid/liquid interfaces. Moreover, most studies are focused on real systems, where large number of interfering compounds is involved. Sabah and Majdan (2009) have studied adsorption of total phosphorus on acid-activated sepiolite and they proposed a mechanism based on hydrogen bonding between the PL molecules and surface silanol groups. Protonation of the silanol groups at slightly acidic conditions and subsequent anion exchange was also considered for uptake mechanism of inorganic phosphates. On the other hand, van Dalen and van Putte (1992) investigated physical degumming with precipitated silica and found that hydratable PLs have higher affinity than the non-hydratable. They also demonstrated linear correlation between the meso-pore surface area and PL adsorption capacity. No detailed mechanism was, however, proposed. Hancer et al. (2002) have experimentally studied adsorption of lecithin and some pure PL on various model surfaces. In all studies, PL are considered physically adsorbed with relatively weak binding on the solid surface.

The objective of this work is to investigate factors affecting phospholipid adsorption on clay surface. For this purpose, adsorption on acid-activated sepiolite was measured at typical oil bleaching conditions as well as in model experiments, where pure PL dissolved in rapeseed oil were used. Acid-activated sepiolite was selected as the adsorbent, because its favorable adsorption properties and it is widely used in refining processes. The adsorbent is a hydrous magnesium silicate clay mineral and most of Mg is removed by acid treatment thereby increasing the porosity and specific surface area. Influence of PL type, ionic form and oil water content was studied. Importance of adsorbate-adsorbent and adsorbate-adsorbate interactions is assessed

by correlating the experimental data with the Moreau isotherm. The results are discussed in view of possible binding mechanisms.

2 Materials and methods

2.1 Materials

Commercial acid-activated sepiolite was used as adsorbent and its porous structure was characterized by means of N_2 adsorption. Most of the pores were found to be in the mesoporous range with an average pore diameter of 25 nm. Specific surface area and porosity of these pores were $176 \text{ m}^2/\text{g}$ and 0.82 mL/g , respectively. Moisture content of 7.6 wt% was found for the air-dry clay as determined by drying overnight at 110°C . Clay obtained at these conditions was stored in a desiccator and it is referred to as dried clay.

Vegetable oils studied in this work are water-degummed camelina oil (CAM) and refined rapeseed oil (RAP). CAM was used as natural source of PL, while RAP was used as oil matrix for adsorption of pure PL. Total phosphorus content was analyzed using inductively coupled plasma spectrometer (ICP-OES, PerkinElmer Optima 7300 DV) and a value of 19.9 mg/kg was obtained for CAM. No phosphorus was detected in RAP. Detection limit for total P was 0.3 mg/kg and the results are estimated to be correct within 5 %. ICP-MS was also used to analyze metals content in the oil and the results are shown in Table 1.

Detailed distribution of the phosphorus compounds in CAM is given in Table 1. The PL were analyzed using a proprietary HPLC–MS method. Main constituents are PA, lyso-phosphatidic acids (LPA) and PC. The chemical

Table 1 Properties of camelina oil

Compound	Content ^a	
	mg/kg	mmol/kg
Total phosphorus	19.9	0.640
Phosphate	0.74	0.024
Phospholipids		
Phosphatidic acids	8.70	0.281
Lyso-phosphatidic acids	1.00	0.032
Phosphatidyl cholines	0.34	0.011
Others	0.95	0.031
Metal cations		
Fe^{3+}	0.12	0.0022
Na^+	0.25	0.011
Ca^{2+}	21.9	0.548
Mg^{2+}	3.6	0.15

^a For phosphorus compounds, all concentrations are in mg P/kg or mmol P/kg

structures are shown in Fig. 1. These three compounds constitute 92.5 % of all CAM PL identified in the analysis.

Water content was determined using Karl Fischer titration and values of 0.04 and 0.02 wt% were obtained for CAM and RAP, respectively.

Pure PL used in this study include dioleoylphosphatidic acid (monosodium salt, >98 %, Sigma-Aldrich) (DOPA-HNa) and dioleoylphosphatidyl choline (>99 %, Avanti Polar Lipids) (DOPC). Moreover, DOPA-HNa was converted to the free acid, DOPA-H₂, using the procedure of van Wijk et al. (1992). Stock solutions were prepared in chloroform or chloroform/methanol mixtures and they were stored at −20 °C.

2.2 Methods

2.2.1 Zeta-potential measurements

Before measurements, suspensions containing 50 g/L of acid-activated sepiolite were prepared in 0.010 M NaCl, ultrasonicated and then solution pH was adjusted. High solids content was found necessary to obtain reproducible results. The supporting electrolyte was added in order to minimize ionic strength effects. The suspension samples were allowed to equilibrate overnight and zeta-potential was measured using Zetasizer Nano ZS (Malvern).

2.2.2 Adsorption measurements

Two sets of experiments were made. In the first series, typical bleaching conditions were used for the camelina oil. 150 g of CAM containing 0.2 wt% of water was put in a 500 mL three-neck bottle which was placed in an electric heating mantle. The oil was mixed with 0.25–2.0 wt% of

air-dry clay and the mixture was kept under reduced pressure (800 mbar). Temperature was controlled at 80 °C and the stirring speed was 270 min^{−1}. The mixture was stirred for 1 h and then water was removed by heating at 105 °C for 30 min at 80 mbar. The solution was then centrifuged and vacuum-filtered through a 0.45 µm membrane filter to get a sample for analysis of total phosphorus (P_{tot}) and metals with ICP-MS.

In the other series of experiments, pure PL were first dissolved in the rapeseed oil pre-equilibrated to water content of 0.02, 0.2 or 1.0 wt%. After addition of water, the oil was mixed with high-speed mixer (Ultra-Turrax) at 8000 min^{−1} for 30 min. Part of the oil was warmed to about 70 °C under nitrogen and PL stock solution was added to obtain a phosphorus concentration of about 100 mg/kg. Exact value was analyzed using ICP-MS. The solvents were evaporated by flushing the warm oil with nitrogen for 1 h. This spiked oil was diluted to desired initial P_{tot} concentrations and suitable amounts of clay were added. The oil/clay mixtures were shaken at least 24 h at 65 °C and then clay was separated from oil by centrifugation. Equilibrium content of P_{tot} was analyzed by means of ICP-MS.

The adsorbed amount q was calculated from Eq. (1) where m_{init} and m_{eq} are the initial and equilibrium oil-phase molalities, while w_{oil} and w_{ads} are the weight of the oil and adsorbent. The PL concentrations studied here are assumed to be below the critical micelle concentration (CMC) and therefore aggregate formation in the oil phase is neglected. Exact values for the present systems are not available but according to Hancer et al. (2002), CMC of hydratable PA-HNa is about 2.5 mmol/kg in a mixture of *n*-hexane and soybean oil. In this study, equilibrium concentration is always less than 0.5 mmol/kg.

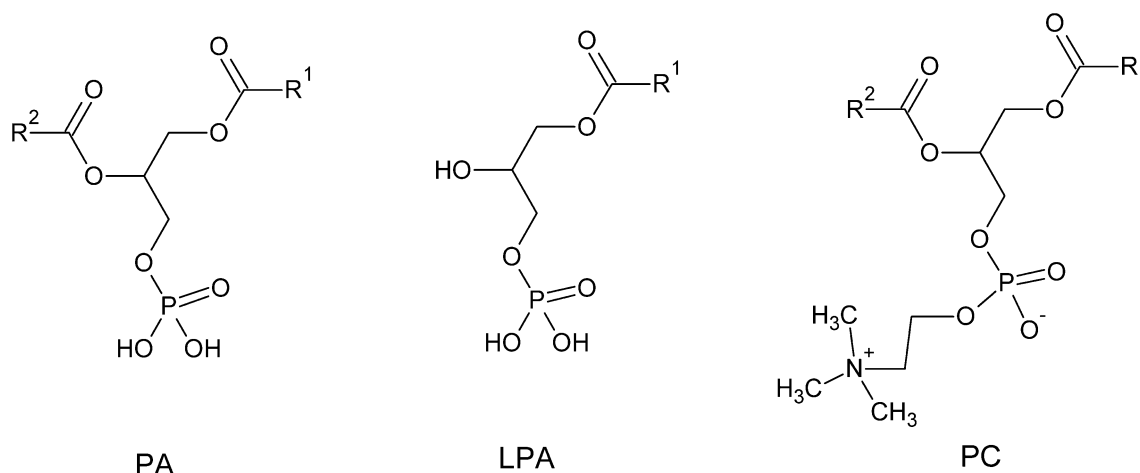


Fig. 1 Chemical structures of phosphatidic acids (PA), lyso-phosphatidic acids (LPA) and phosphatidyl cholines. R¹ and R² are alkyl groups

$$q = \frac{(m_{init} - m_{eq})w_{oil}}{w_{ads}} \quad (1)$$

The data were correlated with the Moreau isotherm equation (Moreau et al. 1991; Gritti and Guiochon 2004) given in Eq. (2) for single-component adsorption. Previously, Langmuir isotherm has been found suitable for adsorption of phosphorus compounds from oil (Sabah and Majdan 2009). Both models are suitable for monolayer adsorption but in contrast to the Langmuir model assuming no lateral interactions, the Moreau model includes parameter I accounting for the adsorbate–adsorbate (A–A) interactions. When the interaction energy ε_{AA} vanishes, $I = 1$ and the two models become equal. For attractive interactions, $I > 1$ and for repulsive interactions, $0 \leq I < 1$.

$$q = \frac{q_{max}[Ka + I(Ka)^2]}{1 + 2Ka + I(Ka)^2} \quad a \approx \frac{m}{m^0} \quad (2)$$

$$K = \exp\left(-\frac{\Delta G_{ads}}{RT}\right), \quad I = \exp\left(-\frac{\varepsilon_{AA}}{RT}\right)$$

In Eq. (2), a is activity, m^0 is unit molality (1 mol/kg), q_{max} is monolayer capacity, K is adsorption equilibrium constant and ΔG_{ads} is Gibbs energy of adsorption. The parameters were estimated using the generalized reduced gradient (GRG) method and goodness of fit was determined by the average relative deviation, ARD, defined in Eq. (3), where N_{dp} is number of data points.

$$ARD = \frac{1}{N_{dp}} \sum_{j=1}^{N_{dp}} \left| \frac{q_{exp} - q_{calc}}{q_{exp}} \right| \times 100\% \quad (3)$$

3 Results and discussion

3.1 Adsorption from camelina oil

In this Section, results obtained with CAM at the bleaching conditions are discussed. Water content of the oil was 0.2 wt% and air-dry clay was used. Adsorption isotherms for untreated CAM in terms of total phosphorus content (P_{tot}) are depicted in Fig. 2. Because of small number of data points, no attempt was made to estimate the adsorption parameters and the solid lines are only to guide the eye.

For comparison, similar isotherms measured for acid-degummed CAM are also shown in Fig. 2. Degumming was made with 0.08 wt% of citric acid at standard conditions and contents of P_{tot} , Ca^{2+} and Mg^{2+} in acid-degummed CAM were 0.40, 0.0325 and 0.012 mmol/kg. Moreover, uptake data for Ca^{2+} and Mg^{2+} are included in order to test the hypothesis that these metals are mainly coupled with the PL. These plots are, however, not

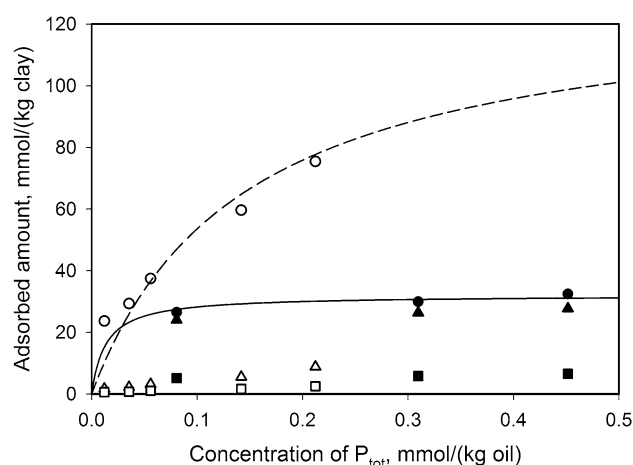


Fig. 2 Adsorption of total phosphorus (circles), Ca^{2+} (triangles) and Mg^{2+} (squares) on sepiolite from camelina oil. $T = 80^\circ C$. Filled symbols and solid line refer to untreated oil, and open symbols and dashed line to acid-degummed oil, respectively. Continuous lines are guide for the eye

isotherms because the adsorbed amounts of metals are plotted against concentration of total phosphorus. Actual isotherms for Ca^{2+} and Mg^{2+} are shown in Fig. 3. Concentrations of Na^+ and Fe^{3+} were much lower (see Table 1) and they are not considered here.

In accordance with earlier studies (van Dalen and van Putte 1992; Hvolby 1971), the amount of P_{tot} adsorbed on the clay strongly depends on the history of the oil and the isotherms for the untreated and acid-degummed oils are thus quite different. Increase of the adsorption capacity in the acid-treated oil stems from transformation of PLs from non-hydratable divalent metal salts to more hydratable free

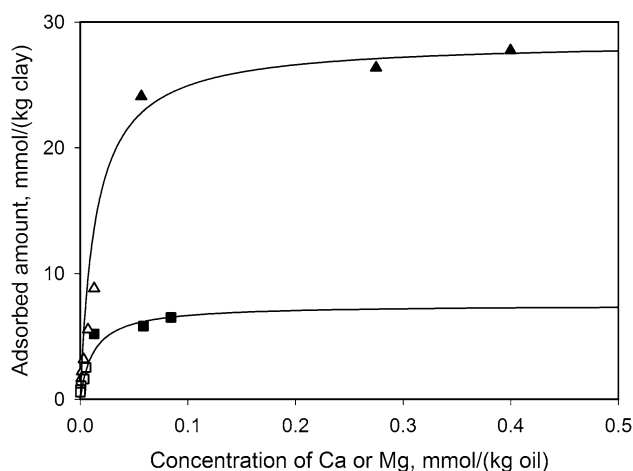
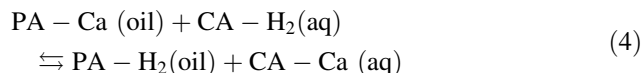


Fig. 3 Adsorption isotherms for Ca (triangles) and Mg (squares) on sepiolite from camelina oil. $T = 80^\circ C$. Filled and open symbols refer to untreated and acid-degummed oil, respectively. Continuous lines are guide for the eye

acids, which have much higher affinity on the adsorbent surface (van Dalen and van Putte 1992; Hvolby 1971; Gutfinger and Letan 1978). Formally this process can be described by Eq. (4), where CA stands for citric acid.



This transformation is clearly seen from Fig. 2. In case of the untreated CAM, sum of adsorbed amounts of Ca and Mg closely matches with that of P_{tot} suggesting that the metal cations are coupled with the adsorbed phosphorus compounds. According to Hvolby (1971), non-hydratable PL remaining after water-degumming are typically composed of Ca and Mg salts of phosphatidic acids (PA).

The data in Table 1 shows that about 90 % of the identified PLs constitute of PAs and LPA, which both are dibasic acids with pK_a values of 3.1 and 8.0 (Marsh 2013) and thus capable to form divalent metal salts with a 1:1 stoichiometry (Cohen and Cohen 1984). For the acid-degummed CAM, on the other hand, most of the adsorbed phosphorus compounds are in forms other than the Ca and Mg salts (see Fig. 2). At least the PAs and LPAs most probably exist in the free acid form.

These data therefore indicate that majority of the PL actually are transformed from their Ca and Mg salts to free acids and the PL uptake on the clay surface increases significantly. However, as shown by the decrease in the initial slope of the isotherms, the affinity of PLs on sepiolite appears to decrease due to this transformation. This point is further discussed in adsorption of pure phosphatidic acids (see Sect. 3.2). It seems also that Ca and Mg remaining in the acid-degummed oil follows the same isotherm as in the original oil and thus behaves similarly in both cases. This conclusion is, however, less obvious than with P_{tot} because of the very low metal concentrations after acid treatment and because complexed/precipitated metal cations are also present in the adsorption system.

Detailed analysis of the data in Fig. 2 is of course complicated by the fact that P_{tot} is not a well-defined compound. As shown in Table 1, only about 55 % of P_{tot} can be ascribed to specific PL, mostly PAs and LPAs. Therefore, the isotherm for PA + LPA was constructed in order to see if there are differences in adsorption of P_{tot} and of the ionizable PL. The results are shown in Fig. 4.

As shown in Fig. 4, the ratio of adsorbed amounts $q_{\text{PA+LPA}}/q_{P_{\text{tot}}}$ was about 0.5, which is close to the ratio in the oil phase, i.e. $c_{\text{PA+LPA}}/c_{P_{\text{tot}}} = 0.55$. It seems therefore that the unidentified fraction in P_{tot} behaves very similarly as the phosphatidic/LPA. Consequently, the above analysis of the P_{tot} uptake data appears reasonable even though the fraction of unknown species is large.

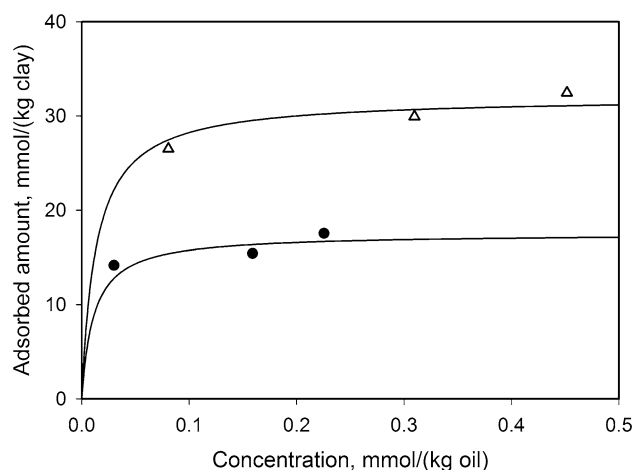


Fig. 4 Adsorption isotherms for PA + LPA (filled circles) and P_{tot} (open triangles) on sepiolite from untreated camelina oil. $T = 80^\circ\text{C}$. Continuous lines are guide for the eye

3.2 Adsorption of pure phospholipids from rapeseed oil

Next, adsorption of individual PL from the RAP matrix was studied. The isotherms of the PL studied here are shown in Fig. 5. These data were measured at oil water content of 0.2 wt% and with air-dry clay. Importance of different interactions was elucidated by correlating the adsorption data using the Moreau equation (Eq. 2) that has been derived for systems including both adsorbent-adsorbate and adsorbate-adsorbate interactions (Moreau et al. 1991). The calculated isotherms are shown in Fig. 5 as solid lines and the estimated parameters are given Table 2.

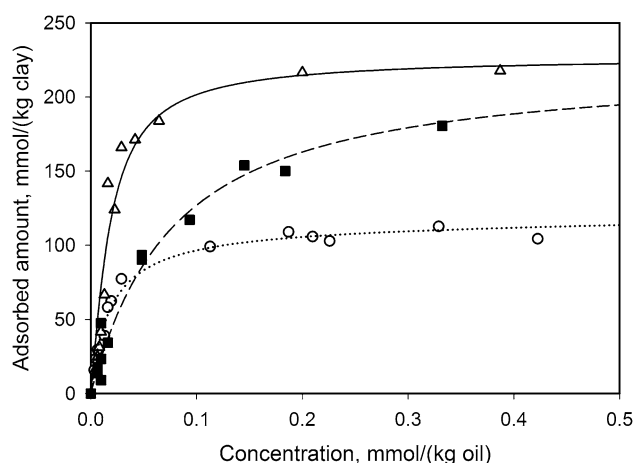


Fig. 5 Adsorption of DOPA-HNa (open circles, dashed line), DOPA-H₂ (filled squares, dashed line) and DOPC (open triangles, solid line) from rapeseed oil on acid-activated sepiolite. $T = 65^\circ\text{C}$, $w_{\text{water}} = 0.2 \text{ wt\%}$. Continuous lines represent Moreau isotherms calculated with parameters of Table 2

Table 2 Moreau parameters for adsorption of pure phospholipids from rapeseed oil (RAP)

Adsorbate	w_{water} (wt%)	q_{max} (mol/kg)	$10^{-3}K$	$-\Delta G_{\text{ads}}$ (kJ/mol)	I	$-\varepsilon_{\text{AA}}$ (kJ/mol)	ARD (%)
DOPA-HNa ^a	0.2	0.2267	27.68	25	0.05751	−12	5.3
DOPA-H ₂	0.2	0.2301	11.97	23	1.046	0.11	5.4
DOPC ^b	0.03	0.2135	42.32	26	3.114	2.8	5.0
DOPC	0.2	0.2276	33.24	26	2.737	2.5	9.5
DOPC	1.0	0.2355	45.77	27	2.064	1.8	6.0

ARD is the average relative deviation defined in Eq. (3)

^a diolelphosphatidic acid

^b diolelphosphatidylcholine

When comparing the results shown in Fig. 5 with those obtained at bleaching conditions (Fig. 2), the adsorbed amounts are similar as with acid-degummed CAM but 3–7 times as high as with untreated CAM. Direct comparison is not possible because of the lower temperature used in adsorption from RAP, although the temperature effect should be quite small (Sabah and Majdan 2009). According to the calculations, all adsorption curves measured at a constant water content of 0.2 wt% can be explained with same maximum capacity $q_{\text{max}} = 228 \pm 2$ mmol/kg (Table 2), which is quite similar to values reported in the literature. Sabah and Majdan (2009) have obtained $q_{\text{max}} = 90$ mmol/kg for adsorption of P_{tot} from acid-degummed rapeseed oil on acid-activated sepiolite at 80 °C. For water-degummed soybean oils, capacities ranging from 25 (Gutfinger and Letan 1978) to 200 mmol/kg (van Dalen et al. 1989) have been found for PL adsorption on commercial bleaching earths.

The saturation capacity q_{max} can also be used to estimate the average area occupied by each adsorbed PL molecule thus giving a measure of the packing density. For DOPC, $q_{\text{max}} = 2.28 \cdot 10^{-4}$ mol/g and $A_{\text{tot}} = 176$ m²/g thus resulting in a molecular area of 1.3 nm²/molecule. This value agrees well with that calculated from the data of van Dalen and van Putte (1992). They have compiled adsorption capacities for silica, silica–alumina and bleaching earths, and found a linear dependence on the specific surface area. The average molecular area calculated from the slope is 1.2 nm²/molecule. These values are less than twice the areas found for PL molecules in ordered mono- or bilayers; Patil et al. (1979) have reported 0.74 and 0.78 nm²/molecule for DOPA-H₂ and DOPA-HNa in monolayers formed at air/water interfaces at 0.017 N/m and 25 °C. Consequently, relatively densely packed and possibly ordered adsorbed layers appear to form on the clay surface. The corresponding values calculated for DOPA-HNa and for the Ca/Mg salts of CAM phosphatidic acids on clay are 2.6 and 9.2 nm²/molecule indicating much lower packing density. The former value is surprisingly large in view of

the moderate expansion observed at air/water interface (Patil et al. 1979). This may be due to the fact that the range of electrostatic interactions is longer in the oil having a dielectric constant of about 3 (Pecovska-Gjorgjevič et al. 2012). The large molecular area of the Ca/Mg salts suggests that no ordered structure is possible and adsorption takes place as isolated PL molecules. According to Patil et al. (1979), DOPA-Ca monolayers at air/water interface are unstable and tend to collapse.

Figure 5 also indicates a very significant difference in adsorption of DOPC and DOPA-HNa. In both cases, a well-defined saturation capacity is found but the value of the former is about twice the value of the latter. Both PL are hydratable and they have same fatty acid composition. Therefore, explanation for the difference can be possibly found in electrostatic interactions. Electrostatic and other specific PL–PL interactions are important in phospholipid mono- and bi-layers in aqueous systems (Pichot et al. 2013). DOPC is an ampholyte and according to Moncelli et al. (1994), $pK_{\text{a},1}$ of the phosphatidic group is about 0.8 suggesting an electrically neutral zwitter-ionic structure, except at very low pH values. The condition of zero net charge is assumed to prevail also in the oil phase. DOPA-HNa, on the other hand, carries negative charge that can increase electrostatic repulsion between neighboring adsorbed PL molecules or between PL and the adsorbent surface. These considerations apply for aqueous systems but in oil phase containing only small amounts of water, other effects like ion-pairing are probably present thus decreasing the effective charge. If present, electrostatic repulsion would most probably decrease packing density in the adsorbed layer.

In terms of the Moreau model parameters, repulsive interactions between adsorbed PL molecules should give a low value for I . On the other hand, decrease in the K value indicates weaker interactions between PL and the clay surface. As seen in Table 2, highest values for both parameters were obtained for DOPC suggesting strongest attractive interactions between PL and clay as well as

between the adsorbed PL molecules. The corresponding values of $-\Delta G_{\text{ads}}$ and $-\varepsilon_{\text{AA}}$ were 26 and 2.5 kJ/mol. The former value includes the enthalpy and entropy effects but their relative contributions cannot be evaluated from the present data. Lateral interaction energies in phospholipid monolayers at air/water interfaces have been reported to range between 3 and 8 kJ/mol (Fornés 1997). For DOPA-HNa, I tends towards zero thus implying strongly repulsive interactions ($-\varepsilon_{\text{AA}} = -12$ kJ/mol) between the adsorbed PL molecules, while only small decrease in the K and $-\Delta G_{\text{ads}}$ values was observed. This result strongly suggests that lateral interactions between the adsorbate molecules are most important factor for packing of the PL molecules on the clay surface.

In adsorption from the camelina oil, even larger difference was observed between adsorption of Ca/Mg salts and predominantly free acid forms (see Fig. 2). If only electrostatic interactions were present, an opposite trend was expected because of more effective charge shielding by the divalent cations than by Na^+ . Therefore, factors other than electrostatic repulsion seem to be more important in this case.

In this study, surface properties of the clay were studied using zeta-potential measurements. In aqueous environment, natural sepiolite is known to have a point of zero charge at about pH 6 (Dikmen et al. 2011). Acid-activation renders, however, the surface sites substantially more acidic and according to the zeta potentials measured here (Fig. 6), the surface is negatively charged at pH values above 2. Because no reliable zeta potentials could be obtained for clay dispersed in the oil, only data measured in aqueous dispersions are shown. The situation in oil/sepiolite system is different because surface charging in practically non-aqueous systems is based on electron transfer

rather than proton transfer. Donor–acceptor properties of the solvent and solid are known to be important factors, and according to Labib and Williams (1984), oxides and mica acquire negative surface charge in most solvents, except for low-donocity hydrocarbons. It seems, therefore, possible that negatively charged sepiolite particles exist also in the oil dispersion and repulsive interactions between PL and adsorbent may contribute to the low uptake of DOPA-HNa.

The electrostatic hypothesis appears to be supported also by the isotherm measured with uncharged DOPA- H_2 . As mentioned in Sect. 3.1, phosphatidic acids are relatively weak acids with $\text{pK}_a \approx 3.1$ in aqueous systems. It is known, however, that the acidity decreases drastically in non-aqueous systems and the undissociated acid is further stabilized because of weak solvation of the ionic species (Sarmini and Kenndler 1999). It may thus be assumed that in the oil phase DOPA- H_2 carries no negative charge. The mono-layer capacity approached the value of DOPC, while the equilibrium constant K (initial slope of the isotherm) was distinctly lower for DOPA- H_2 (see Table 2) and $-\Delta G_{\text{ads}}$ was 23 kJ/mol. At the same time, the value of I decreased and situation, where practically no lateral interactions are present ($I = 1$), was approached with $I = 1.064$ and $-\varepsilon_{\text{AA}} = 0.11$ kJ/mol. Similar trend was found also in uptake of P_{tot} from camelina oil (see Fig. 2), although the number of data points was too small for unambiguous conclusions. Reasons for this behavior are not known. Although these considerations are more qualitative than quantitative, it seems that both PL–clay and PL–PL interactions are operative in adsorption on acid-treated clay and their relative importance depends on the PL structure.

3.3 Influence of water on PL adsorption

Effect of water contained in the system was studied using DOPC as adsorbate. The isotherms obtained at different RAP water contents are shown in Fig. 7. Air-dry clay with $w_{\text{water}} = 7.6$ wt% was employed, except for the lowest oil water content where dried clay was used. The continuous lines were calculated with Eq. (2) using the estimated parameters given in Table 2.

It is obvious from Fig. 7 that water dispersed/dissolved in the oil phase affects only slightly adsorption of DOPC. A small increase in the saturation capacity was observed with increasing water content but in the range normally used in vegetable oil processing, the influence is insignificant. Added water may react with the surface groups thus altering the donor–acceptor properties and, consequently, surface charging. Labib and Williams (1987) have shown, however, that water added in non-aqueous dispersions of various solids affects only slightly the zeta potential and no

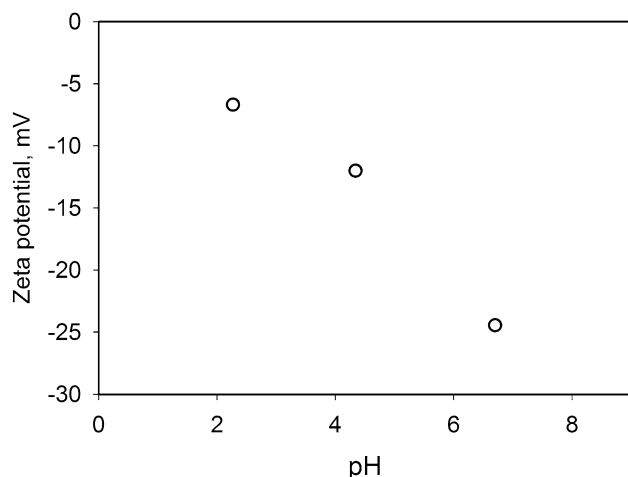


Fig. 6 Zeta-potential of the acid-treated sepiolite used in this study. $T = 22$ °C. $c_{\text{NaCl}} = 0.010$ mol/L

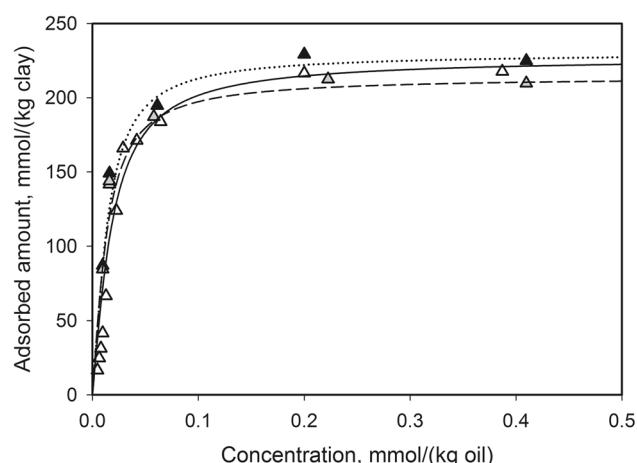


Fig. 7 Effect of oil water content on adsorption of DOPC from rapeseed oil on acid-activated sepiolite. $T = 65\text{ }^{\circ}\text{C}$. $w_{\text{water}} = 0.03$ (shaded triangles, dashed line), 0.2 (open triangles, solid line) and 1.0 wt% (filled triangles, dotted line). Solid lines represent Moreau isotherms calculated with parameters of Table 2

charge reversal has been observed for acidic oxides like silica.

As discussed above, DOPC is a hydratable PL but similar trend has been observed earlier (van Dalen and van Putte 1992) also in adsorption of non-hydratable PLs from water-degummed soybean oil on precipitated silica.

4 Conclusions

Adsorption of natural PL from camelina oil and of spiked dioleoyl phosphatidic acid (DOPA) and phosphatidylcholine (DOPC) from rapeseed oil has been studied using acid-activated sepiolite as adsorbent. We have shown the following.

- Neutral PL adsorb on clay as densely packed monolayers with a packing density of about half the value found in ordered mono- and bilayers of aqueous systems.
- Both PL-clay interactions and lateral interactions between the adsorbed PL molecules appear to be important. Substantially lower adsorption capacity was found for the negatively charged DOPA-HNa than for the neutral DOPA-H₂ or DOPC suggesting that repulsive electrostatic interactions between DOPA-HNa and clay as well as between the adsorbed DOPA-HNa molecules are present. Calculations with the Moreau isotherm suggest that the latter factor is more important.
- Water content of oil or clay plays not an important role in PL adsorption.

Acknowledgments Financial support from TEKES, Finland is gratefully acknowledged. The authors also thank Neste Oil Corp. for analysis of the samples.

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