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Separation of cis/trans geometrical fatty acid isomers by silver-exchanged zeolite Y

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ABSTRACT

The separation of cis and trans isomers is relevant for biological and nutritional applications, silver-exchanged zeolite Y was prepared and applied for the treatment of mixtures of cis and trans geometrical isomers of mono- and polyunsaturated fatty acid methyl esters (FAMEs). cis FAMEs were adsorbed into zeolite with a high degree of selectivity (cis/trans ratio in the range of 1.9–3.2). The effectiveness was due to the synergism of the π-complexation between the silver ion and the double bonds and the different FAME structures trapped into the zeolite cages. Some indication of the complex stabilities came from theoretical studies using unsaturated lipids. A prototype cartridge was also designed for application in the fractionation of cis/trans FAME mixtures.

1. Introduction

Double bonds in natural fats are predominantly in the cis configuration, and most of the structural and metabolic activities of mono- and polyunsaturated fatty acids (MUFAs and PUFAs) are based on this geometric requisite.[1] A great deal of interest arose from the fact that trans fatty acid isomers can be present in foods, in particular in industrialized countries, since fats and oils are manipulated through the partial hydrogenation or deodorization processes,[2] or undergo frying process at high temperatures.[3] In this case, trans structures can be both geometrical and positional isomers. It is now demonstrated that trans fatty acid isomers have harmful effects on health, being risk factors for cardiovascular diseases, heart attack and impairment of fetal and infant growth.[4–8] After these studies and the increase of public concern, a new regulation in USA has established that by 2006 foods must show the trans content in the nutritional facts.[9] Recently, another piece of information came from the studies on lipid double bond isomerization catalyzed by free radical species, which established that the formation of trans lipid isomers can occur in living organisms and, in this case, only geometrical isomers can be formed, with the position of the double bonds identical to the natural cis lipids, but with the opposite geometry.[10]

Separations as well as analytical techniques are in continuous development to examine cis/trans mixtures, due to the strong interest involving food industries. Also for biological studies in lipidomics, these improvements are necessary, because not only the total trans isomer content is important,[11] but also the type of each isomer.[12] The cis/trans FAME separation based on argentation techniques is well known.[13] It involves the reversible reaction of unsaturated organic molecules with transition metals, such as silver, and the formation of a polar charge-transfer complex. Two types of interactions occur simultaneously: a sigma bond between the occupied π-orbitals of the double bond and the empty s-orbitals of the Ag ion, together with a π-acceptor back-bond between the empty unoccupied π^*-orbital of the double bond and the occupied d-orbitals of the silver ion.[14] The methylene-interrupted cis fatty acid methyl esters (FAMEs) are held more strongly, and this effect has been successfully used in TLC and HPLC analyses, impregnating the silica gel stationary phase with a silver nitrate solution in organic solvents. In the case of arachidonic acid, the argentation TLC method was expedient in the identification of the mono-trans isomer fraction.[15] The argentation technique has been applied in solid phase extraction (SPE) columns with stationary phases like silica gel, or a strong cation exchange (SCX) phase such as a bonded-sulfonic acid phase, where silver ions are anchored through electrostatic interactions.[16] Actually the latter system has become commercially available in the form of a cartridge containing 750 mg of the silver phase, with the efficiency of separation for 1 mg of FAMEs.[17]

In this paper, we used silver-exchanged zeolite Y as the stationary phase for the cis/trans mixture of FAME separation. Zeolites, which are mixed silicon/aluminum-based porous materials,

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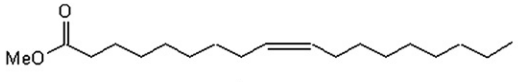
catalyze reactions[18,19] by confining the substrates within 'active site' cavities.[20] We reasoned that zeolites with appropriate cage dimensions of ca. 7–13 Å,[21] such as faujasites (MX, MY, M=cation) would be particularly well suited for our purpose. As far as zeolites are concerned, their structure as crystalline microporous aluminosilicates accommodating metal ions (Na, Ag) in their pores[22,23] is useful for adsorption processes, based on both van der Waals and electrostatic interactions, linking the sorbate to the sorbent, whereas the ions in the cages give also a dimensional limitation to the pores. Only one report in a patent describes the use of sodium ion-containing zeolites (Y-zeolites) for fatty acid isomer separation, which gave a selectivity trans/cis ratio higher than 1, when the adsorbent is used as stationary phase for column chromatography and *n*-hexane as the mobile phase.[24] Ag-exchanged zeolite Y has been reported for industrial purification of 1-butene by removal of 1,3-butadiene traces.[23] To date, there is no information on a system combining the silver π-complexation and the adsorbent capability/pore diameter of zeolites, applied to FAME.

In detail, in this paper a thorough study of silver-exchanged zeolite Y in the separation of mixtures of cis/trans geometrical isomeric mono- and di-unsaturated FAMEs is reported. For comparison the efficiency of the zeolite NaY separation was evaluated under identical conditions. We anticipated that silver-exchanged zeolite Y shows a preference for encapsulating cis isomers, which corresponds to an enrichment of trans FAME in the fluid phase. A theoretical approach to study the stability of cis and trans isomer complexes with silver and sodium cations was also carried out, giving some basic explanation for the different adsorbent behavior with and without silver ions.

2. Results and discussion

2.1. Adsorption studies of cis and trans monounsaturated fatty acid methyl esters into AgY

The use of the sodium zeolite Y (NaY) for fatty acid isomer separation was reported in a patent described with a selectivity ratio trans/cis ratio higher than 1.[24] Silver-exchanged Y-zeolite (AgY) can be prepared by a reported procedure but no information was available on its use for FAME isomer separation. However, an interesting synergy between the silver ion-double bond complexation and the adsorbent pore dimension (for Y-zeolite it is known that the largest pore has a 7.4 Å diameter) could be envisaged.[21] AgY-zeolite was first tested with monounsaturated substrates, using a 1:1 geometrical isomer mixture of fatty acid methyl esters (FAMEs), such as 9-*cis*-octadecenoic acid methyl ester (*cis*-$C_{18:1}$, Δ9, methyl oleate, **1**) and 9-*trans*-octadecenoic acid methyl ester (*trans*-$C_{18:1}$, Δ9, methyl elaidate, **2**).



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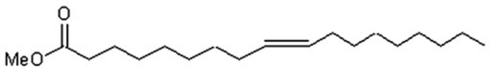

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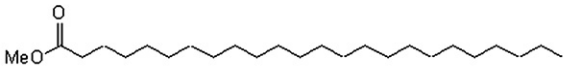

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In particular, dried and activated AgY was added to a solution containing an equimolar amount of **1** and **2** in *n*-hexane, stirring the slurry and withdrawing samples of the *n*-hexane phase at different time intervals, for GC analysis and control the FAME isomeric composition. Actually, it is known that for sodium Y-zeolite (NaY), the ratio of 0.1 mmol of substrate per each gram of adsorbent is used.[19] In the present work, we kept this ratio, using a total of 0.01 mmol of the isomeric mixture with 100 mg AgY. A lower ratio was also tested for comparison, using 0.01 mmol FAME isomers with 30 mg AgY.

The *n*-hexane solution containing the fatty acids contained also a known amount of a 24-carbon atom saturated fatty acid, tetracosanoic acid, as the methyl ester (**3**). This compound cannot be complexed by silver ions, or trapped into AgY cavities, and it served as the internal standard to monitor the variations of the geometrical cis/trans isomer molar concentrations in a time interval. A 60 min period was monitored at a constant temperature of 20 °C. In Figure 1, the results for the two different amounts of zeolite are shown. Starting from a 1:1 cis/trans mixture, it is clear that the cis isomer (**1**) is incorporated with a higher affinity, lowering its concentration in the *n*-hexane phase compared to the corresponding trans isomer (**2**). After ca. 10 min the separation process nearly reached the plateau, with a constant isomer composition. In the presence of a 30 mg amount of AgY, less material was adsorbed (see molarity values on the *y* axis of Fig. 1), as it could be expected. However, the efficiency of the separation was not affected (Table 1).

The substrate adsorbed in the AgY was also examined. Methanol allowed the fatty acids to be desorbed from zeolite with the best efficiency. After methanolic treatment, zeolite was filtered off and the organic phase was evaporated to afford a solid residue (Ag-complexes of the FAME isomers). This residue was washed with an aqueous solution of NH_4OH (5% v/v) followed by extraction with *n*-hexane. GC analysis gave the cis/trans fatty acid methyl ester ratios trapped into zeolite. In Table 1 the cis/trans ratios, determined in *n*-hexane and in zeolite, are shown for the two different AgY amounts used. In all cases, the unsaturated fatty acid ratios were higher than 1, indicating the preferred adsorption of cis isomer. From Table 1 it can be appreciated that the cis-isomer inclusion in 30 mg adsorbent resulted to be better than in 100 mg. The fatty acid–adsorbent weight proportion corresponded to about 1:10 ratio. It is worth noting that in the case of the Ag-SPE cartridges, a 1:750 ratio is reported, that shows the effectiveness of the Ag-zeolite material in comparison with other Ag-exchanged phases.[17] Using 100 mg of AgY, the trans isomer started to be enclosed, and the cis/trans ratio consequently was reduced.

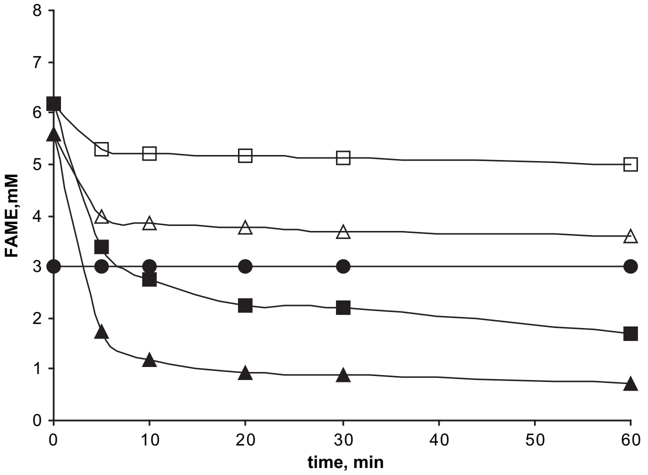


Figure 1. Time profiles of FAME concentrations found in *n*-hexane, after mixing a 6 mM *n*-hexane solution of a 1:1 mixture of methyl oleate 1 (triangles) and methyl elaidate 2 (squares) with 30 mg AgY (open symbols), or with 100 mg AgY (solid symbols), respectively. Solid circles (●) correspond to the internal standard, tetracosanoic acid methyl ester (3), present in a solution at a concentration of 3 mM.

Table 1
cis/trans Ratios of MUFA (ca. 3 mg, 0.01 mmol from a 6 mM solution) adsorbed into AgY in function of the reaction time[a]

Time (min)	cis/trans ratio adsorbed into	
	30 mg AgY	100 mg AgY
5	1.7	1.5
10	1.9	1.4
20	1.9	1.3
30	1.9	1.3
60	1.9	1.3

[a] The ratios were determined by gas chromatography. The error was ±1%.

Looking at the isomer content remaining in solution, the cis/trans ratio is expected to be <1, with a trans isomer prevalence, with the best results achieved with 100 mg AgY. Table 1 also shows that after 10 min the ratios did not change significantly, the cis isomer remaining trapped in different amounts depending on the adsorbent quantity. Several factors are synergistic in this process: (a) the different π-complexation process between a silver ion and the double bond of the cis and trans isomers, (b) the conformation of the FAME and their complexes interacting with the zeolite cages and (c) other factors (temperature, concentration) influencing the thermodynamic equilibrium of the processes.

For this reason, we repeated the experiments at two different temperatures of 0 °C and 50 °C, and the results are shown in Figure 2. At low temperature (0 °C) the adsorption remained almost stable after 30 min, whereas at 50 °C it was not able to reach the plateau after 180 min. A slight effect was seen at this temperature, being the cis/trans isomer ratio adsorbed in the AgY (30 mg) of 2.2, whereas the behavior at 50 °C was similar to that observed at 20 °C.

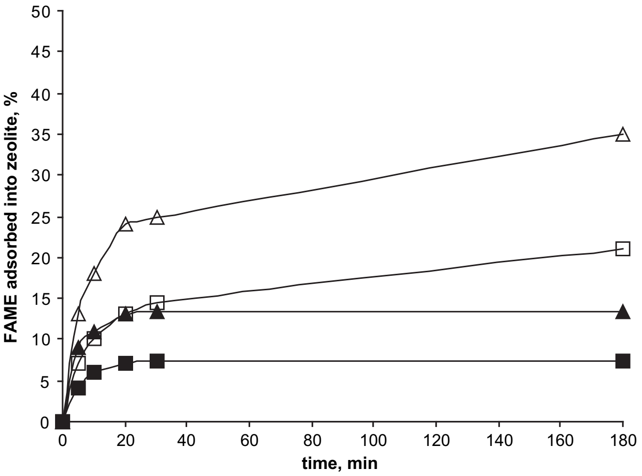


Figure 2. Time profiles of the adsorbed methyl esters starting from a 50:50 mixture of methyl oleate **1** (triangles) and methyl elaidate **2** (squares) in *n*-hexane (0.01 mmol) added with 30 mg AgY at 50 °C (open symbols) and at 0 °C (solid symbols).

Next we examined the adsorption in Ag-zeolite when a single isomer is present. A solution of the pure cis or trans monounsaturated fatty acid methyl ester (**1** or **2**) was added with 30 mg of AgY in *n*-hexane (always corresponding to 0.01 mmol of substrate). GC monitoring showed that inclusion of the cis isomer was more efficient (Fig. 3).

Calculating the cis/trans isomer ratios from the experiments with the single isomer, we obtained the values shown in Table 2, that are in the range of 2.7–3.2. We recall that, starting from the equimolar mixtures of cis and trans isomers, the range was 1.7–1.9 (Table 1). This indicates that the interaction with adsorbent is influenced by the two isomers together, and this is probably due to the contribution of the Ag-complexation process. An indication of the role of the Ag-complexation was addressed later by theoretical calculations.

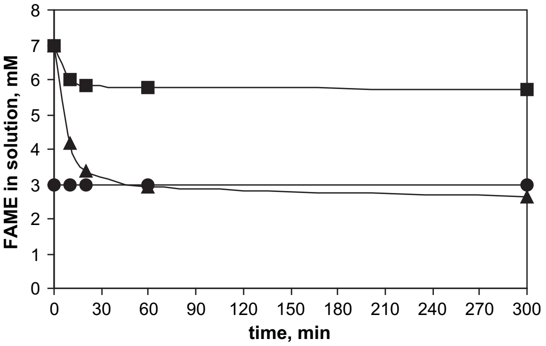


Figure 3. Time profiles of methyl oleate (**1**, ▲) and methyl elaidate (**2**, ■) concentration in *n*-hexane upon addition of AgY (30 mg) to a solution containing the single isomer. Solid circles (•) correspond to tetracosanoic acid methyl ester **3** used as the internal standard.

Table 2
Relative amounts of methyl oleate **1** and elaidate **2** (0.01 mmol) adsorbed into AgY (30 mg) in the absence of the other isomer[a]

Time (min)	% cis	% trans	cis/trans ratio
10	41	15.5	2.7
20	52	18	2.9
60	59	18.5	3.2
300	63	20	3.1

[a] The relative amounts were determined by gas chromatography. The error was ±1%.

Further data about isomer interference during the π-complexation were obtained by the addition of one isomer to a suspension where the silver-exchanged zeolite Y has already enclosed the other geometrical isomer. Two zeolite suspensions (30 mg) were added with one isomer (ca. 7 mM) and stirred until a constant lipid concentration was observed (t=300 min). After this time, an amount of the other isomer (4 mM) was added to the slurry, and the isomeric composition present in solution was monitored by GC. The time courses are shown in Figure 4A and B. Figure 4A shows that the added trans isomer was able to interact with Ag-zeolite and the cis isomer was displaced from the zeolite, going into solution. It is worth recalling that tetracosanoic acid methyl ester was used as the internal standard, since its concentration in *n*-hexane remains constant. The displacement occurred also in the case of the cis isomer added to the slurry of zeolite enclosing the trans isomer (Fig. 4B). From quantitative analysis, in the two experiments it was found that by the addition of cis isomer, a 55% of the trans isomer was displaced from the zeolite, whereas by adding the trans isomer only a 25% of the cis isomer was displaced from the AgY-zeolite. These experiments indicated that the behavior of cis and trans isomers is different, with the trans isomer interacting less with the zeolite. This favors the hypothesis that the bent structure of the cis isomer and its stronger interaction in the zeolite cages play a role in the cis-isomer selectivity, and explains the efficiency of the silver zeolite as material for separation compared to other solid phases.

Finally, we compared the separation given by the sodium zeolite Y (NaY) under the conditions used with the Ag-exchanged zeolite Y. NaY (26 mg) was used with 0.01 mmol of the cis/trans monounsaturated isomers. After 180 min the system reached a constant concentration, which again corresponded to the preferential adsorption of the cis isomer affording a cis/trans ratio of 1.7. This value is lower than the corresponding value measured in the case of AgY (1.9, cf. Table 1), but it indicates that, under our experimental conditions, the simple zeolite structure also has cis selectivity. We also checked the behavior with only one monounsaturated FAME, in the absence of the other isomer, and we calculated the cis/trans ratio to be 1.4, much lower than that observed using AgY (cf., 3.1 in Table 2). This result gave support to the hypothesis that in the Ag-

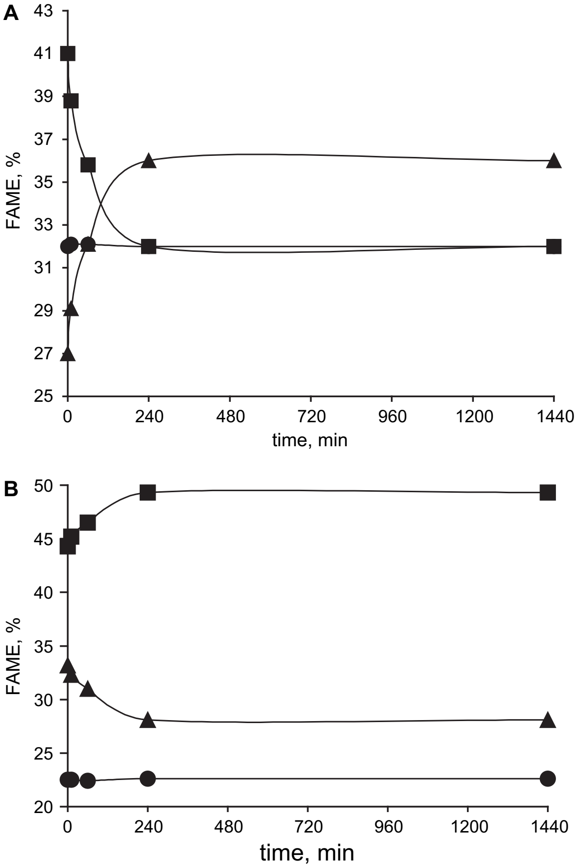
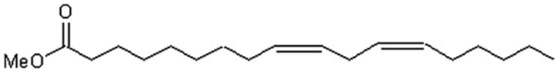


Figure 4. Relative percentages of cis (methyl oleate,▴) and trans (methyl elaidate, ■) isomers detected in *n*-hexane, after addition of methyl elaidate (Fig. A) and methyl oleate (Fig. 4B).

exchanged zeolite Y the interaction with the pores is combined with the Ag-complexation of double bonds, resulting in a more efficient separation.

2.2. Separation of geometrical isomers of linoleic acid methyl ester

The mixture of di-unsaturated lipid isomers was also attempted using AgY. For this reason, linoleic acid methyl ester (9,12-octadecadienoic acid, LAME) isomers were considered as an example for di-unsaturated lipids. The mixture used for separation contained methyl esters of 9-*cis*,12-*cis*-octadecadienoic acid (*cis*,*cis*-$C_{18:2}$ Δ9,12, methyl linoleate, **4**), 9-*trans*,12-*cis*-octadecadienoic acid (*trans*,*cis*-$C_{18:2}$ Δ9,12, **5**), 9-*cis*,12-*trans*-octadecadienoic acid (*cis*,-*trans*-$C_{18:2}$ Δ9,12, **6**), and 9-*trans*,12-*trans*-octadecadienoic acid (*trans*, *trans*-$C_{18:2}$ Δ9,12, methyl linolelaidate, **7**) in 32%, 15%, 15%, and 38%, respectively.



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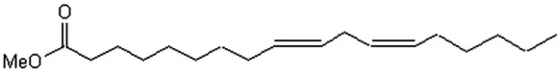

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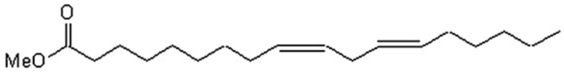

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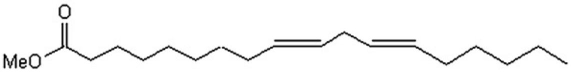

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This mixture (3 mg, 0.01 mmol) was dissolved in 1 mL of *n*-hexane and stirred with two different amounts of AgY, 30 mg and 100 mg. The separation processes were monitored by GC analysis under conditions where the four isomers were perfectly resolved, and the time profile of the reaction with 30 mg AgY is shown in Figure 5. Again, the higher affinity of the AgY for the cis isomer (**4**) compared to the other isomers was observed. It is interesting to note that the *cis*,*trans*- and *trans*,*cis*-LAME isomers (**5** and **6**) are adsorbed in equal amounts into zeolite, and they are better enclosed than the all-trans isomer (**7**).

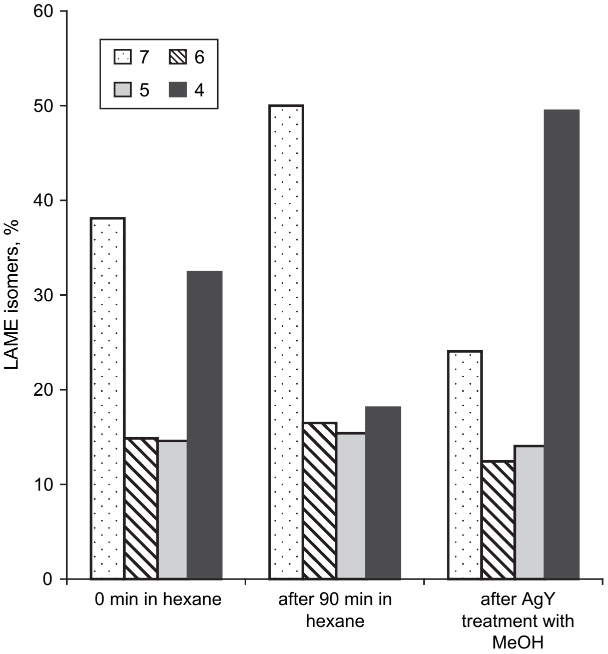


Figure 5. Behavior of LAME isomers in the absence and presence of 30 mg of AgY. Initial ratio of **4**:**5**:**6**:**7** was 3.2:1.5:1.5:3.8, respectively.

Calculation of the all-*cis*-LAME/all-*trans*-LAME ratio gave the better value after 20 min of ca. 2.9 (Table 3). The cis/trans ratio resulted better than the corresponding monounsaturated compounds (see Table 1), suggesting that a stronger Ag-complexation is involved in di-unsaturated cis substrates. Indeed, it is known that 1,3-buradiene is selectively adsorbed over 1-butene.[23] With 100 mg AgY the all-cis/all-trans ratio worsened, affording after 20 min the value of 2.0. As previously described with monounsaturated compounds, a quantitative recovery of all the fatty acid methyl ester amount was achieved by methanol washing. In Figure 5 the isomer composition of the methanolic phase is reported.

Table 3
All-*cis*-LAME (**4**)/all-*trans*-LAME (**7**) encapsulation ratio into AgY-zeolite

Time (min)	All-*cis*-LAME/all-*trans*-LAME[a]	
	With 30 mg AgY	With 100 mg AgY
10	2.8	2.1
20	2.9	2.0
60	2.4	1.6
300	2.2	1.4

[a] The ratios were determined by gas chromatography. The error was ±1%.

The efficiency of the cis/trans di-unsaturated fatty acids separation by Ag-exchanged Y-zeolite was finally tested in a sample where the amount of trans isomers is $<10\%$. This condition reproduced the situation of foods or biological samples, where the natural cis compound is prevalent. A mixture containing the all-cis (**4**), the two mono-trans (**5** and **6**), and the all-trans (**7**) isomers, in 92.1, 1.7, 1.7, and 4.5%, respectively, was used. For this separation a prototype cartridge for small scale analysis was realized using the adsorbent (in the quantity of 50 or 150 mg) divided in three portions and alternated in a glass support (Pasteur pipette) with anhydrous Na_2SO_4, as the drying agent (Fig. 6). The cartridge was eluted with *n*-hexane, and then the above described FAME mixture (0.01 mmol corresponding to ca. 3 mg) dissolved in 0.5 mL of *n*-hexane was loaded, including tetracosanoic acid methyl ester (**3**) as the internal standard and allowed to interact for 30 min. Subsequent elution with *n*-hexane (1.5 mL), allowed a first fraction to be obtained containing 70% of the starting lipid mixture with an isomer composition similar to the starting material. Interestingly, methanol washing (4 mL) gave a fraction corresponding to the remaining 30% of the starting material, and having an isomer composition of all-cis (**4**), the two mono-trans (**5** and **6**), and the all-trans (**7**) isomers in 97.3, 0.8, 0.7, and 1.2%, respectively. Therefore, starting from a mixture with ca. 8% of trans-containing isomers, the fraction remained in the zeolite was essentially enriched of the cis isomers. This procedure was repeated two more times and the results were similar.

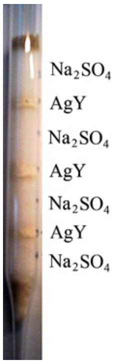


Figure 6. Prototype cartridge for small scale analysis with alternated layers of Ag-zeolite and anhydrous Na_2SO_4.

2.3. Theoretical studies of complexation Na^+ and Ag(I) with various geometrical isomers $C_{18:1}$ Δ9 and $C_{18:2}$ Δ9,12 FAMEs

Theoretical calculations of the interaction between metal cation and lipids were also carried out in order to support some of the present results. As a model of complexation was pitched upon a chelate-like structure, because such type of complexes for lipids is energetically much more stable than structures formed due to interaction only with carbonyl group or double bound.[25] Conformational analysis was made for all studied complexes and the conformers with lower formation enthalpies selected only. These structures are shown in Figure 7 and the results of calculation, various geometrical isomers $C_{18:1}$ Δ9 and $C_{18:2}$ Δ9,12 FAMEs are listed in Table 4. In the case of 9,12-octadecadienoic acid methyl esters complexes with smaller rigs have less stability for Na^+ and Ag(I), up to 10 kcal/mol (see Table 4). For this reason such structures will not be considered.

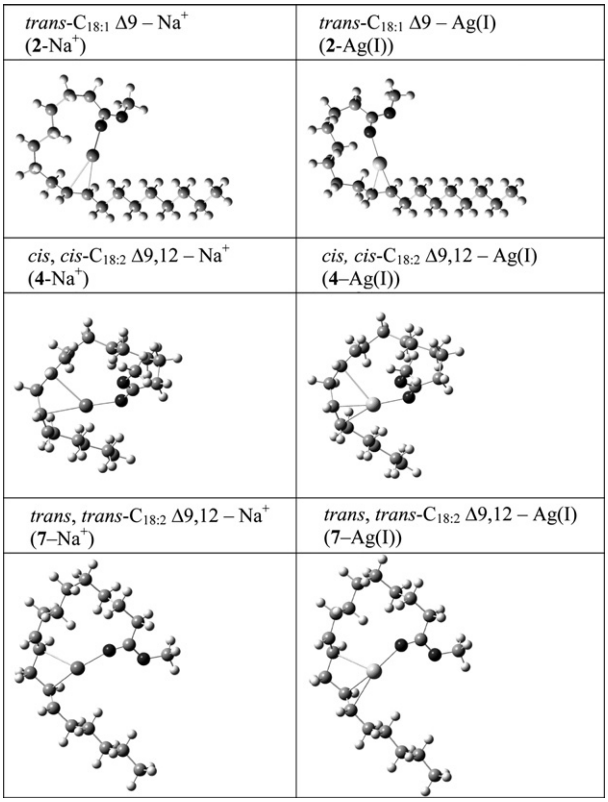


Figure 7. Optimized structure of the complexes Na^+ and Ag(I) with *trans*-$C_{18:1}$ Δ9 and *trans,trans*- and *cis,cis*-$C_{18:2}$ Δ9,12 methyl esters at the RHF/6-31G(d,p) level, except Ag(I), which was treated with the SDD pseudo potential.

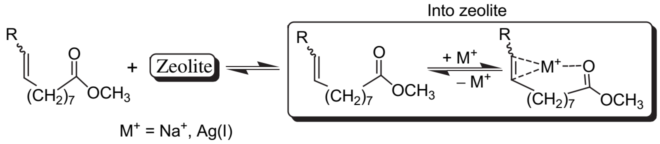
As follows from results of calculations of thermodynamic parameters of complexes, a cis-isomer complex is more stable than trans-, for both sodium and silver. However, from Table 4 it is clear that Ag-complexes (cis- or trans-) are more stable than

Table 4
Theoretical enthalpies,[a] entropies and free energies[a] for the complexes of silver and sodium cations with various geometrical isomers of $C_{18:1}$ Δ9 and $C_{18:2}$ Δ9,12 FAMEs at the RHF/6-31G(d,p) level (for Ag(I) was used the SDD pseudo potential)

Ligand	Interacted atom	Metal	$\Delta E_{\text{complex.}}$, kcal/mol	ΔS_{298}, cal/(mol·K)	ΔH_{298}, kcal/mol	ΔG_{298}, kcal/mol	$\delta\Delta G_{298}$, kcal/mol
cis-$C_{18:1}$ Δ9	9, 10	Na^+	−50.0	−25.9	−50.2	−42.5	0.0
trans-$C_{18:1}$ Δ9	9, 10	Na^+	−49.9	−28.2	−50.4	−42.0	0.5
cis-$C_{18:1}$ Δ9	9, 10	Ag^+	−54.3	−30.8	−54.6	−45.4	0.0
trans-$C_{18:1}$ Δ9	9, 10	Ag^+	−53.1	−30.9	−53.6	−44.4	1.0
cis,cis-$C_{18:2}$ Δ9,12	10, 12	Na^+	−58.4	−26.8	−58.9	−50.9	0.0
trans, trans-$C_{18:2}$ Δ9,12	10, 12	Na^+	−58.4	−30.3	−58.9	−49.9	1.0
cis, cis-$C_{18:2}$ Δ9,12	10, 12, 13	Ag^+	−63.6	−31.1	−64.0	−54.7	0.0
trans, trans-$C_{18:2}$ Δ9,12	10, 12, 13	Ag^+	−62.3	−32.5	−62.7	−53.0	1.7
cis, cis-$C_{18:2}$ Δ9,12	9, 10	Na^+	−49.3	−28.8	−49.7	−41.1	0.0
trans, trans-$C_{18:2}$ Δ9,12	9, 10	Na^+	−48.9	−30.7	−49.3	−40.2	0.9
cis, cis-$C_{18:2}$ Δ9,12	9, 10	Ag^+	−53.8	−32.4	−54.2	−44.5	0.0
trans, trans-$C_{18:2}$ Δ9,12	9, 10	Ag^+	−52.4	−34.9	−52.8	−42.4	2.1

[a] Corrected for basis set superposition error.

Na-complexes of ∼3 to 4 kcal/mol. This indicated that by changing from sodium to silver as the cation of the adsorbent, it achieves an additional contribution of stability that ameliorates the efficiency of the material, as also demonstrated experimentally. A reasonable mechanistic explanation for the studied system can be described by Scheme 1.



Scheme 1. Proposed equilibrium pathways of the lipid adsorption into metal-exchanged zeolite pore.

Two equilibria occur in this adsorption process. The first one can describe the adsorption of fatty acid methyl ester (FAME) into zeolite and the second one formation of the complex between lipids and the metal cation. In order to elucidate, which of the two equilibria is the candidate for the separation of the lipid isomers, we compare the values calculated for the K_{cis}/K_{trans} ratio from the computation data and the experimental results (where K is dissociation constant of complex FAME with Ag^+). The theoretical equilibrium ratios were calculated using the definition $K_{cis}/K_{trans}=\exp(-\delta\Delta G^{compl}/RT)$, $\delta\Delta G^{compl}=\Delta G_{trans}-\Delta G_{cis}$, with ΔG_{trans} and ΔG_{cis} the free energy of complexation cis and trans isomers with metal cation. And the experimental equilibrium ratios were calculated using the following equation:

$$\frac{K_{cis}}{K_{trans}} = \frac{[cis]_{\infty}^{free}\left[Ag^{+}\cdots trans\right]_{\infty}}{[trans]_{\infty}^{free}\cdot\left[Ag^{+}\cdots cis\right]_{\infty}}$$

where $[cis]_{\infty}^{free}$ and $[trans]_{\infty}^{free}$ concentration of cis and trans isomer in solution after the establishment of equilibrium; $[Ag^{+}\cdots cis]_{\infty}$ and $[Ag^{+}\cdots trans]_{\infty}$ concentration of the complexes from cis and trans isomers, respectively. As shown in Table 5, the theoretical values are in good agreement with experimental, despite the fact that calculations were carried out for the gas phase and without taking into account the complexation between metals and surface of zeolite pore. Assuming that these factors are similar for cis and trans isomer, the error for the K_{cis}/K_{trans} measurement is negligible. From Table 5 it is clearly shown that the first equilibrium is faster than the second one (Scheme 1), and the complex with the metal cation is the key factor for the separation of cis/trans isomers.

Table 5
Theoretical and experimental values of K_{cis}/K_{trans} for the complexes of silver and sodium cations with geometrical isomers of $C_{18:1}$ Δ9 and $C_{18:2}$ Δ9,12 FAMEs at 298 K

Complex	Theoretical K_{cis}/K_{trans}	Experimental K_{cis}/K_{trans}
1-Na^+ versus **2**-Na^+	0.43	0.42
4-Na^+ versus **7**-Na^+	0.18	—
1-Ag(I) versus **2**-Ag(I)	0.18	0.14
4-Ag(I) versus **7**-Ag(I)	0.06	0.09

3. Conclusions

Silver-exchanged zeolite Y is a promising material for separation of saturated versus unsaturated substrates, and in particular for the first time it has been used to fractionate geometrical cis and trans FAME isomers. The cis isomers are adsorbed in the AgY with a high degree of selectivity, which increases by the number of double bonds (ca. cis/trans=3.1/1). The performance of zeolites as adsorbents for unsaturated substrates is greatly ameliorated by the combination of physical adsorption with the silver complexation. Silver zeolite cartridges can be successfully applied in several case of di-unsaturated fatty acids purification, with a performance superior to the actually commercially available SPE phases. Further developments can be foreseen also in lipidomics, such as fractionation of FAME mixtures derived from biological samples usually rich in cis isomers, in order to obtain differently enriched cis and trans isomers, respectively, and facilitate further characterization by other techniques.

4. Experimental section

4.1. Preparation of silver-exchanged zeolite Y (AgY)

The sorbent was the silver-exchanged zeolite Y (AgY). Ion exchange of zeolite was performed in aqueous solution. Faujasite was selected as the zeolite framework structures for ion exchange, because of its large pore aperture (7.4 Å), large pore volume (0.48 mL/mL) and wide range of SiO_2/Al_2O_3 ratios. The molecular form of Na zeolite Y that was used in this study is $Na_{56}(AlO_2)_{56}(SiO_2)_{136}\times H_2O$(NaY), with a Si/Al ratio of 2.43. Silver ion-exchanged process was carried out by using excess [fivefold cation exchange capacity (CEC)] of 1 M $AgNO_3$ at room temperature for 24 h.[23] Because of the known higher selectivity of Ag^+ compared to that of Na^+,[26,27] this procedure ensured 100% exchange for NaY (Si/Al) 2.43.[28] After the exchange, the zeolite suspension was filtered and washed five times with deionized water, until no free ions were present in the filtered water (checked by the presence of precipitate upon treatment with Cl^-). The solid was dried first at room temperature and atmospheric conditions in a dark area for 2 h, and after in vacuum for 10 h. Before each experiment the zeolite amount was activated at 120 °C under vacuum (∼7 mbar).

4.2. Materials

The methyl esters of 9-*cis*-octadecenoic acid, 9-*trans*-octadecenoic acid, 9-*cis*,12-*cis*-octadecadienoic acid, 9-*cis*,12-*trans*-octadecadienoic acid, 9-*trans*,12-*cis*-octadecadienoic acid, 9-*trans*, 12-*trans*-octadecadienoic acid, and tetracosanoic acid were commercially available and used without further purification. *n*-Hexane and methanol (HPLC grade) were used without purification.

4.3. General methods

GC analysis for the determination of the isomeric ratio of the methyl esters of the unsaturated fatty acids was performed for each experiment at different time intervals. An Agilent 6850 gas chromatograph equipped with a flame ionization detector and a DB-23 column (90% bis-cyanopropyl-10% phenylcyanopropyl polysiloxane capillary column; 60 m, 0.25 mm i.d., 0.25-μm film thickness) was used. Temperature started from 190 °C, held for 15 min, followed by an increase of 30 °C/min up to 220 °C, held for 15 min, followed by a second increase of 30 °C/min up to 250 °C. Helium was the carrier gas at a constant flow of 29 psi.

4.4. Cis–trans separation using silver-exchanged zeolite Y

To a slurry containing from 30 to 100 mg of activated AgY (or NaY, or HY) in 1 mL of *n*-hexane containing 3 mM of the methyl ester of tetracosanoic acid as the internal standard, 1.6 mL of a 6 mM cis/trans FAME solution in *n*-hexane (corresponding to ca. 3 mg; 0.01 mmol) was added and the heterogeneous slurry was stirred for 1–5 h. At different time intervals, stirring was stopped and an aliquot of the supranatant was withdrawn for GC analysis and determination of the isomer content. When the isomer ratio became constant, the slurry was filtered for zeolite separation. The zeolite was then suspended in methanol (2×1 mL) for 20 min, then filtered, and the methanolic phases were collected and evaporated to afford a solid residue, corresponding to the Ag-complexes of the unsaturated methyl esters. The residue was added to *n*-hexane and treated with 5% aq NH_4OH solution (1 mL). The organic phases were collected, dried over anhydrous Na_2SO_4, and examined by GC to give the fatty acid fraction composition encapsulated in the zeolite.

4.5. Computations

All model complexes were studied using ab initio RHF calculations with the 6–31G(d,p) basis set for all atoms except Ag, which was treated with the SDD[29] pseudo potential. Vibrational frequencies were also obtained at this level. The nature of all stationary point species was verified by calculating the vibrational frequencies.[30] Basis set superposition errors are wherever required accounted for by the counterpoise method.[31] Graphics of computed theoretical structures, orbital and electron density map, are rendered using the MOLDEN[32] and ViewMol3D.[33] All calculations were performed using the Gaussian 03 suite of programs.[34]

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