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Application of Langmuir–Blodgett technology for the analysis of saturated fatty acids using the MALDI-TOF mass spectrometry

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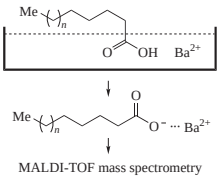
A new procedure for the analysis of saturated fatty acids by the MALDI-TOF method was developed using Langmuir–Blodgett technology and myristic, palmitic and stearic acids as examples. The advantages of proposed procedure include simplicity, rapidness, and high sensitivity of the analysis.



The Langmuir–Blodgett technologies provide an opportunity to obtain water-insoluble monomolecular layers (MML) of organic amphiphilic molecules[1] and are widely applied in various fields of science and technology.[2,3] For example, adducts of organophosphorus compounds with blood proteins can be isolated from the collapsed MML of trivalent metal (Fe, La) salts from biological samples, which is of great importance in organophosphorus compound intoxication diagnostics, especially in retrospective analysis;[4] and nickel stearate monolayers show good results in the dieldrin determination in water, milk and blood serum.[5] Already formed monolayers are currently used for practical purposes as either collapsed or transferred onto a solid support ones. The procedure for obtaining such structures can be useful for analytical chemistry. The MML formation occurs at the interface between an aqueous phase containing metal ions and an organic phase containing an amphiphilic compound (Figure 1).

The amphiphilic compounds include fatty acids (FAs), which are structural components of lipids and are present in biological objects, both in free and bound forms. The analysis of free FAs, including saturated ones, in lipid fractions of blood plasma is of great diagnostic importance for the identification of various pathologies in clinical studies.[6,7] Moreover, some works have been reported on the analysis of FAs in the follicular fluid, wherein their concentration was comparable to that in the blood plasma, or even lower.[8,9] FAs are in most cases identified by the GC-MS method in the form of volatile derivatives.[10] However, their derivatization is undesirable because of significantly complicated analysis and separation of the free FA from the bound one.[11] During the MML formation, the carboxyl group of FA submerged in the aqueous phase interacts with the metal ion under conditions that do not cause the cleavage of lipids, which consequently prevents the participation of bound FAs in the MML formation. At pH > 4, the carboxyl groups dissociate, which leads to the salt formation on the entire surface of the monolayer.[12,13]

We have previously reported[14] that the signal corresponding to iron distearate is reliably detected by MALDI-TOF mass spectrometry of monolayer based on iron(III) stearate dissolved in acetonitrile. Note that in the case of organic phase consisting of the FAs mixture, the trivalent metal ions form mixed salts, which complicates an interpretation of the analysis results. However, an application of a divalent alkaline-earth metal (*e.g.*, barium) solution as an aqueous phase would provide MMLs consisting predominantly of the monocarboxylates of such a metal,[15] which

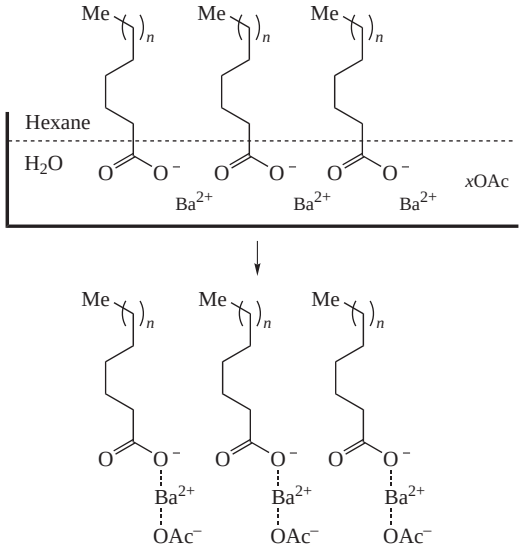


Figure 1 Formation of the monolayer.

could significantly simplify the mass spectra interpretation. In addition, a characteristic isotopic pattern of barium allows one to easily identify the compounds containing this element.

Taking into account the above points, this work was aimed at the development of a new approach to the analysis of saturated FAs using Langmuir–Blodgett technology.

Three saturated FAs were chosen as the model objects: myristic, palmitic, and stearic.† The obtained mass spectra are shown in Figure 2. According to our expectations, the signals corresponding to barium monomyristate (m/z = 365.106 for $C_{13}H_{27}COOBa^+$), barium monopalmitate (m/z = 393.139 for $C_{15}H_{31}COOBa^+$), and barium monostearate (m/z = 421.168 for $C_{17}H_{35}COOBa^+$) were observed in the mass spectra of samples obtained by dissolving based on them monolayers, while m/z and isotopic pattern

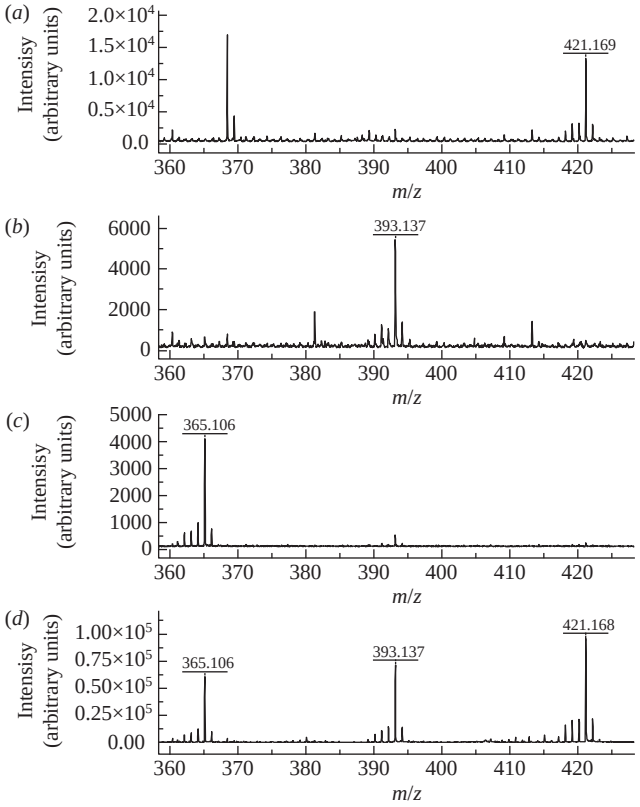


Figure 2 Mass spectra of redissolved monolayers obtained by layering the FA solutions in hexane onto an aqueous support containing Ba^{2+} ions: (*a*) barium stearate; (*b*) barium palmitate; (*c*) barium myristate; (*d*) a mixture of barium fatty acid salts.

† The myristic (M3128, Sigma-Aldrich), palmitic (43051, Sigma-Aldrich), and stearic (S4751, Sigma-Aldrich) acids were used. The experiment was performed using a Langmuir–Blodgett bath (1.8 dm^3), S monolayer bounded by barriers of 7 dm^2, and 10 mM aqueous barium acetate (243671, Sigma-Aldrich). A hexane (34859, Sigma-Aldrich) aliquot of 10 μl containing one of the listed FAs was layered on the aqueous support at a concentration of 1 mg ml^{-1}. Hexane was completely evaporated and the reaction was finished in 2 min; after that the monolayer was collapsed, transferred to a microtube, and dissolved in acetonitrile (50 μl, 100%) (271004, Sigma-Aldrich) with added 0.1% of trifluoroacetic acid (TFA) (302031, Sigma-Aldrich). TFA was used for a more effective destruction of the monolayer and also for the transfer of carboxylic acids salts in the solution. The procedure was carried out alternately for all the selected acids, and for their mixture with the concentration of each acid of 0.25 mg ml^{-1}. The solution aliquot of 1.5 μl was applied to the target, and saturated ammonium sulfate solution (1 μl) (A2939, Sigma-Aldrich) in aqueous acetonitrile (20%) was added. The MALDI-TOF mass spectrometry analysis [Ultraflex (ultrafleXtrem) TOF/TOF instrument, Bruker] was performed in the reflectron mode of the positive ions detection in the mass range of 150–1000 Da (ion source 1, 20 kV; ion source 2, 17.9 kV; lens, 7 kV; PIE, 120 ns; laser repetition rate, 2000 Hz).

completely corresponded to the calculated ones [Figure 2(*a*)–(*c*)]. The same result was obtained in the analysis of a monolayer formed from a mixture of acids [Figure 2(*d*)]. Note that ammonium sulfate was used for the ionization only as an additive to other matrices in analysis of compounds such as peptides,[16] carnitine, acetylcarnitine, glycerophosphocholine,[17] and glycolipids.[18] The physical and chemical properties of this salt allowed one to assume its practical effectiveness in the pure form as a matrix for MALDI-TOF mass spectrometry analysis of FA salts. Ammonium sulfate sublimates upon exposion to the laser, which facilitates the analyte transfer into the gas phase. Moreover, the appearance of interfering signals in the mass range corresponding to masses of analytes is not expected due to the low molecular weight of this salt. As mentioned above, the main samples, where FA detection is practically needed, are plasma and blood serum.

Therefore, an aqueous solution of human serum albumin with a concentration of 100 mg ml^{-1} was chosen as the biological model, which corresponds the total average concentration of proteins in the blood plasma.‡ In the mass spectrum of the dissolved monolayer, signals corresponding to all three barium monocarboxylates were detected, but the compound identification by isotopic distribution failed because of a low intensity of the barium monomyristate signal. Hence, the procedure of sample application to the target had to be optimized. A solution of barium acetate with concentration of 10 mg ml^{-1} was used as a new matrix. Under such conditions, the signals corresponding to all three barium monocarboxylates were reliably detected in mass spectra [Figure 3(*a*)]. In contrast, the application of standard matrices, such as 2,5-dihydroxybenzoic acid, α-cyano-4-hydroxycinnamic acid, *etc*. did not provide the detection of the test compounds. Based on the acquired results, we proposed the new

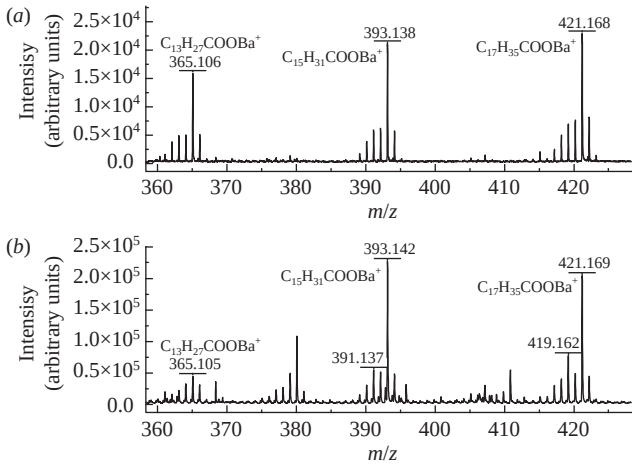


Figure 3 Mass spectra of redissolved in acetonitrile multilayers obtained by layering on an aqueous substrate containing barium acetate: (*a*) hexane after extraction from a solution of human serum albumin (100 mg ml^{-1}) containing myristic, palmitic and stearic acids at a concentration of 5 nmol ml^{-1}; (*b*) hexane after extraction from the follicular fluid.

‡ The commercial (A3782, Sigma) human serum albumin was used. An acetonitrile solution containing the mixture of myristic, palimitic, and stearic acids was added to the protein solution (1 ml) until the final concentration of 5 nmol ml^{-1} was reached. The mixture was incubated for 2 h and extracted with hexane (200 μl). A glass Petri dish (0.12 dm^3) was used as the bath; the concentration of barium acetate in the aqueous phase, as in the previous experiment, was 10 mg ml^{-1}. The extract aliquot of 150 μl was applied to an aqueous support; 2 min later the monolayer was collapsed by moving barriers, transferred into a microtube and dissolved in acetonitrile (50 μl, 100%) with 0.1% of TFA added. The solution was dried with a vacuum centrifuge and the precipitate was redissolved in acetonitrile (10 μl, 100%) with 0.1% of TFA added. The MALDI-TOF mass spectrometry analysis was carried out under the same conditions.

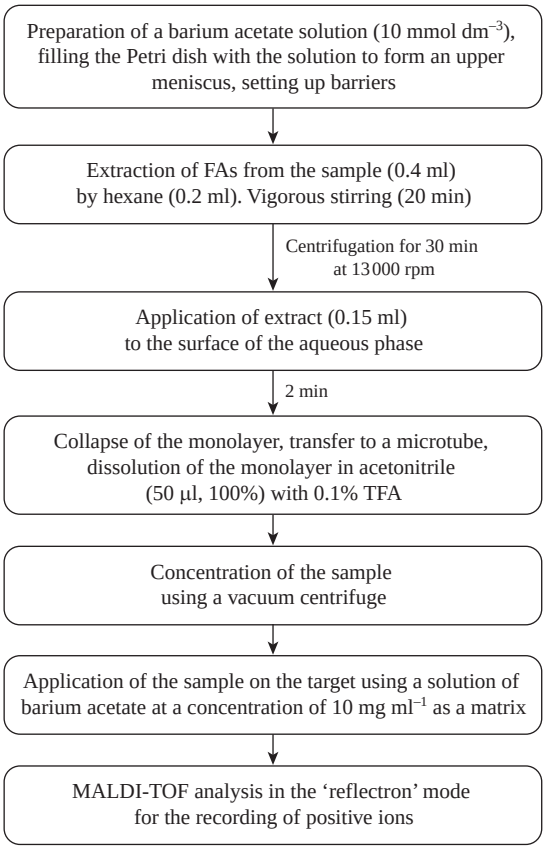


Figure 4 Procedure for the analysis of fatty acids by the MALDI-TOF method using the Langmuir–Blodgett technology.

procedure for the analysis of saturated FAs by the MALDI-TOF mass spectrometry (Figure 4).

The proposed procedure was verified by the analysis of FAs in the follicular fluid. The sample (400 μl) was extracted by hexane (200 μl). The signals in the mass spectrum corresponding to barium monocarboxylates and the results of MS-MS analysis (Table 1) indicated the reliability and effectiveness of the developed approach [Figure 3(*b*)]. The higher intensity of peaks with *m*/*z* 391.137 and 419.162 compared with the calculated one suggested a presence of palmitoleic and oleic acids in the sample, respectively.

Table 1 Results of MS-MS analysis of barium monocarboxylates extracted from follicular fluid.

Fatty acid	Ion	*m*/*z*	*I* (%)
Stearic acid	$C_{17}H_{35}COOBa^+$	421	4
	$C_{16}H_{33}CH{=}COBa^+$	404	29
	$C_{16}H_{33}CH{=}C^+$	250	5
	$C_3H_2BaO_2^+$	208	9
	Ba^{2+}	138	33
Palmitic acid	$C_{15}H_{31}COOBa^+$	393	35
	$C_{14}H_{29}CH{=}COBa^+$	376	5
	$C_{14}H_{29}CH{=}C^+$	222	1
	$C_3H_2BaO_2^+$	208	7
	Ba^{2+}	138	100
Myristic acid	$C_{13}H_{27}COOBa^+$	365	100
	$C_{12}H_{25}CH{=}COBa^+$	348	17
	$C_{12}H_{25}CH{=}C^+$	194	3
	$C_3H_2BaO_2^+$	208	6
	Ba^{2+}	138	64

In summary, we have proposed a new approach to the analysis of saturated FAs by the MALDI-TOF mass spectrometry using Langmuir–Blodgett technologies. The method was verified using myristic, palmitic and stearic acids, illustrating the possibility to detect the composition of FAs using MMLs of barium monocarboxylates. The advantages are high sensitivity, reproducibility, speed, and usability. This allows one to apply the developed procedure for the rapid screening of FAs in biological samples of various nature.

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