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Analysis of complex mixtures recovered from space missions Statistical approach to the study of Titan atmosphere analogues (tholins)

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Abstract

To study Titan, the largest moon of Saturn, laboratory simulation experiments have been performed to obtain analogues of Titan's aerosols (named tholins) using different energy sources. Tholins, which have been demonstrated to represent aerosols in Titan's haze layers, are a complex mixture, resulting from the chemical evolution of several hydrocarbons and nitriles. Their chromatographic analysis yields complex chromatograms, which require the use of mathematical procedures to extract from them all the information they contain. Two different chemometric approaches (the Fourier analysis approach and the statistical model of peak overlapping) have been successfully applied to pyrolysis–GC–MS chromatogram of a tholin sample. Fundamental information on the mixture's chemical composition (number of components, m) and on the separation system performance (separation efficiency, σ) can be easily estimated: the excellent correspondence between the data calculated by the two independent procedures proves the reliability of the statistical approaches in characterizing a tholin chromatogram. Moreover, the plot of autocorrelation function contains, in a simplified form, all the information on the retention pattern: retention recursivities can be easily singled out and related to specific molecular structure variations. Therefore, the autocorrelation function (ACF) plot constitutes a simplified *fingerprint* of the pyrolysis products of tholins, which can be used as a powerful tool to characterize a tholin sample.

Keywords: Titan's atmosphere; Aerosols; Statistical analysis; Autocorrelation function; Autocovariance function; Tholins; Volatile organic compounds

1. Introduction

Gas chromatography (GC) plays a predominant role in solar system explorations, in particular in space research related to exobiology, i.e., the investigation of history and abundance of biogenic elements, which throws light on the necessary conditions for prebiotic chemical evolution and origin of life [1]. The study of Titan, the largest moon of Saturn, is very relevant to exobiology, since space

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missions (Voyager 1, 1980 and Voyager 2, 1981) showed that its atmosphere is one of the most dense and complex in the Solar System, and with a chemistry that could be similar to the Earth's primeval one. Titan atmosphere is composed by nitrogen with a few percent of methane and many hydrocarbons as minor constituents, with the same nature as organic compounds involved in terrestrial prebiotic chemistry [1–4]. The Voyager data also revealed an abundance of aerosols and cloud droplets in the atmosphere, which masks the surface of the satellite and permits adsorption of cosmic rays [4]. In addition to remote sensing observation in space missions (properly treated with theoretical modelling) laboratory simulation experiments have been performed, where analogues of Titan's aerosols (named tholins) were obtained using different energy sources [5,6]. Independent investigation procedures, like pyrolysis, elemental analysis, optical and spectroscopic observations give consistent results with photochemical models and confirm that laboratory analogues represent aerosols in Titan's haze layers with sufficient accuracy [1–6].

All the reported results [1,5,6] show that the solid phase synthesized during simulation experiments is a complex oligomer, resulting from the photochemical evolution of several hydrocarbons and nitriles. Tens of compounds (>70) have been identified by GC–mass spectrometry (MS) analysis after pyrolysis of the sample: C_1–C_8 hydrocarbons (saturated and unsaturated), C_1–C_4 nitriles, dinitriles and O-organics at trace level [1,2,5]. A chromatographic analysis of such multicomponent mixtures yields complex chromatograms characterized by a random crowd of peaks allocated along the chromatographic space: some regions are full of overlapped peaks and some are nearly empty [7,8]. Such behaviour derives from the mixture complexity and the separation system performance as well as from their mutual interaction. This situation results in a drop of the analytical information hidden by the involved signal and peak overlapping. Two different procedures can be applied in order to extract the most analytical information from the complex chromatogram. The first approach is the use of two-dimensional separation systems (LC–GC, GC–GC, GC×GC) to increase resolution power and reduce peak overlapping or hyphenated techniques [LC with UV–Vis detection, LC–nuclear magnetic resonance (NMR), GC–MS] to identify eluted components through spectral information [8,9]. The second procedure is a mathematical approach to deconvolve incompletely resolved peaks and interpret the chromatogram, in order to extract from it all the information it contains, that is ''decoding'' the complex chromatogram [10–24]. This last approach can be coupled to the first one to quantify overlapped peaks [11,21] or applied to conventional GC instruments fulfilling the severe constraints of space missions [1–3].

The present paper describes the application of two statistical approaches [12–24] to a complex GC–MS chromatogram obtained from a tholin sample: the reliability of the obtained results is verified as well as the usefulness of the procedure to extract important information for the study of Titan's environment.

2. Experimental

2.1. Tholins preparation

The reactor consisted of a Pyrex glass cylinder (100×75 mm) with a volume of 0.47 l equipped with a high vacuum stopcock to facilitate the connection of the reactor to the manifold. A tungsten rod (120×1 mm; 99.99%) was located at the centre of the cylinder and acted as the internal electrode to which a regulated high-voltage d.c. power supply with reverse polarity was connected [5,6]. A stainless steel plate (200×80×2 mm thickness) which grounded through a small tungsten rod (30 mm) internally covered the glass cylinder. With this array, coaxial corona discharges are generated. A flow of an N_2–CH_4 (90:10) mixture with a pressure of 800 mbar was irradiated by the positive coronas for 720 min. To collect the tholin samples, the reactor was put inside a glove box which was filled with N_2, to prevent contamination: the top of the reactor could be opened to introduce collector plates or to sample the aerosol analogues, without any contamination.

2.2. GC–MS analysis

The sample analysis was performed using a HP gas chromatograph 5890 series (Hewlett-Packard, Palo Alto, CA, USA) interfaced in series with a HP

quadrupole mass spectrometer (5989 series) equipped with electron impact and chemical ionization detection modes. The GC–MS system was coupled with a HP pyrolizer (Hewlett-Packard). After pyrolysis (at 750°C for 1 min), the gas sample was directly injected into the GC system using an automatic six-port gas sampling valve with a 2-ml gas loop. The column used was a 25 m×0.32 mm I.D. PoraPlot Q fused-silica with a 2.5 m gas particle trap. The following temperature programme was used: 60°C for 2 min, then 10°C/min to 240°C, hold for 30 min then 10°C/min to 250°C and hold for 9 min. The total GC run was 60 min, the last 10 min being used to clean the column at 250°C.

3. Theory

A chemometric approach is based on the *observable* attributes that can be directly measured form the experimental chromatogram; on these bases, a proper statistical model is built up to describe the chromatogram, regarded as a statistical ensemble [7,8,21,22]. Through mathematical tools the average properties of the model can be statistically estimated, i.e., all the *intrinsic* attributes inherent to the type of mixture and to the separation system, which allow a precise and detailed description of the complex chromatogram [7,12–24]. The most relevant parameters are the following.

(i) Complexity of the mixture, represented by the number of single components, m, present in the mixture. From the experimental chromatogram the number of detectable peaks, p, can be determined: some peaks are pure (formed by one single component per peak) but some peaks derive by the overlapping of two or three single components. Therefore p value is always lower than m value and it does not represent the real complexity of the mixture: the loss of analytical information is represented by the separation degree $\gamma=p/m$ and is proportional to the degree of overlapping present in the chromatogram [7,22].

(ii) Efficiency of the separation system, represented by peak standard deviation σ. The experimental determination of this parameter is difficult, due to peak overlapping, and time consuming, due to the large number of peaks present in the complex chromatogram. The chemometric approach makes it possible a direct statistical evaluation of the mean σ value as an average value computed on all the peaks present in the chromatogram. This parameter is fundamental to detect the presence of effects increasing peak width, i.e., strong retention determining peak tailing or overloading effects; it can also be used to select the best temperature programme conditions determining constant peak width [12–16,18,19].

(iii) Presence of an ordered structure in the retention pattern: it is related to specific structure variations in the separated compounds that determine constant retention time increments, repeated in the chromatographic space [16–19]. Usually a multicomponent chromatogram displays a crowd of peaks randomly distributed in the chromatographic space, so that an ordered structure, if present, is hidden in it and it is very troublesomely detected; the chemometric approach is able to magnify the ordered structure and single it out from the ''disordered forest'' of the random peaks.

Among the several mathematical methods to deal with this problem [7,8,10,21], some of the authors proposed two distinct and independent approaches [12–24].

3.1. Fourier analysis approach

This approach is based on the study of the autocovariance function (ACVF) that can be experimentally computed from the experimental chromatogram acquired in digitized form and whose expression was mathematically derived in terms of the chromatographic parameters [12–21]. It represents the short and long term correlation between subsequent chromatographic peaks observable in the chromatogram. In the complete procedure, the experimental ACVF is compared to theoretical ones using non-linear fitting algorithms: the parameters of the best fitting model describe the experimental chromatogram [12–17]. Moreover, a simplified procedure was also developed to estimate the chromatographic parameters, m and σ [18–20]: it is based on a simple graphic inspection of the ACF plot (ACF is autocorrelation function, i.e., ACVF where all the data are normalized to the ACVF value computed at time 0). The first part of the ACF plot – 0–4σ

interdistance values corresponding to mean peak width – looks like half a Gaussian peak whose properties are averaged values on all the peaks present in the chromatogram: from it the mean σ value, describing the mean separation performance, is obtained. ACVF value at time 0 is related to the number of single components m present in the mixture, which can be easily estimated. The region of the ACF plot with interdistance values higher than 4σ, contains information on the order present in the retention pattern: it shows some positive peaks if an ordered retention pattern exists in the chromatogram, that is, if peaks appear at a repeated interdistance as a sequence (representing an homologous series) or at constant interdistances located in different parts of the chromatogram (i.e., a functional group increment) [16–20].

3.2. Statistical model of peak overlapping: pulse point SMO

This approach describes the complexity of a multicomponent chromatogram in terms of probability functions derived from two types of distribution [23,24]: the first is the interdistance model (IM), which describes the single peaks position along the retention time axis [8,9,22], the second is the abundance model (AM), which represents the peak abundance distribution. Starting from the *observable* parameters corresponding to the integrator output (i.e., retention time and area/height for each peak) a critical interdistance value x_0 (related to the required chromatographic resolution) is selected and used to count the peaks and evaluate the observed mean peak area. Different x_0 values can be selected and many ln $\overline{y_{obs}}/x_0$ couples obtained (with an experimental limit due to the instrumental minimum interdistance between two resolved peaks, specific for the integrator software). Different theoretical models have been developed (based on different IM and AM functions) in order to describe the great variability of experimental chromatograms. In the simplest and most general case (corresponding to a random retention time distribution), the relationship of ln $\overline{y_{obs}}$ vs. x_0 is linear [23,24]: the experimental points can be fitted by a straight line, whose slope represents a statistical estimation of m, the number of single components. The specific feature of the experimental points contains information on the retention pattern [23].

4. Results

Fig. 1 shows the GC–MS chromatogram of the pyrolyzed tholin sample. Using a resolution value $R_s = 0.5$, corresponding to peak maximum detection, 50 peaks can be counted; on the basis of their full-scan mass spectra 41 compounds can be identified without any ambiguity: a list of retention time and molecular structure is reported in Table 1.

4.1. Fourier analysis approach

ACVF was computed on the tholin chromatogram (Fig. 1) and plotted versus time span, representing the interdistance between subsequent chromatographic peaks. Fig. 2 shows the first part of the autocovariance function (ACVF) plot. From the value of ACVF at time 0 a number of single components $m = 72$ was estimated. By a simple graphic inspection of this part of the ACVF plot, the half-height peak width $d_{1/2}$was determined and from it the mean σ value was estimated using the simple equation:

$$\sigma = \frac{d_{1/2}}{1.665} \quad (1)$$

A value of 3.5 s was obtained. Also using the complete procedure, based on non-linear fitting, statistically comparable values were obtained ($m = 70$, $\sigma = 3.6$ s). The high σ value, if related to the capillary GC conditions used, suggests the presence of large peaks: a double peak is present at 7 min, corresponding to water and ammonia (Table 1) which are strongly retained on the stationary phase. It must be underlined that the ACVF procedure is very efficient in revealing the presence of any effect determining peak broadening, even if it is present in a few peaks in the chromatogram.

The central part of the chromatogram (13–30 min region reported in the enlarged detail in Fig. 1) contains the most relevant peaks in order to characterize a tholin sample [2,5]. The ACVF procedure was applied to this part (ACF plot in Fig. 3): a value of 2.7 s is obtained for mean σ, revealing that all the peaks are well-shaped in this region of the chromato-

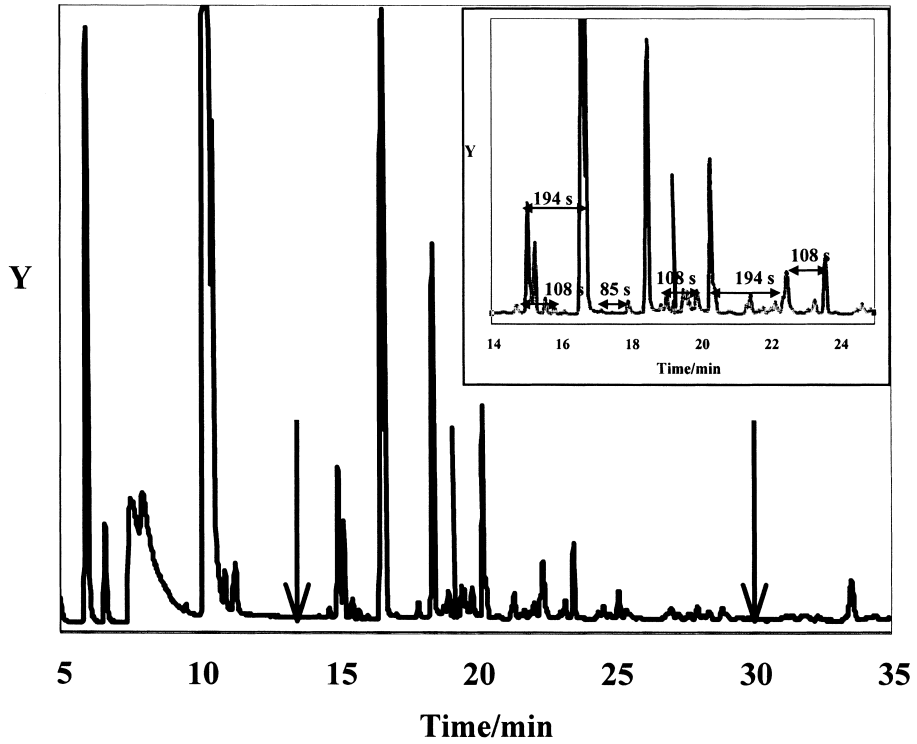

Fig. 1. GC–MS chromatogram of the pyrolyzed tholin sample. Enlarged detail: 13–30 min region where repeated interdistances are marked by arrows.

gram. Some positive peaks appear in the ACF plot at interdistance values of 85, 108, 194 s: this means that some peaks are located at these interdistances, as shown by arrows in the expanded chromatogram in the detail in Fig. 1. Such constant interdistance repetitivities can be related to specific molecular structure effects, on the basis of the molecular structure of the identified chromatographic peaks (Table 1). The lower interdistance value (85 s, arrows in detail of Fig. 1) was found for compound pairs containing the same number of carbon atoms, and can be related to isomerization effects (e.g., compound. 26 vs. 22, compound. 39 vs. 38. in Table 1) the higher retention increments at 108 and 194 s (arrows in detail of Fig. 1) were identified as resulting from the addition of a methyl group to the molecule, since they correspond to compound pairs differing by one carbon atom. In particular, the retention increment of 108 s is related to the CH_2 increment in nitriles (e.g., compound 19 vs. 18, compound 27 vs. 23, compound 29 vs. 25 in Table 1), while that of 194 s corresponds to a CH_2 addition to hydrocarbon structure in aromatic/unsaturated molecules. (e.g., compound 13 vs. 10, compound 21 vs. 17, compound 37 vs. 31 in Table 1) Therefore a simple inspection of the ACF plot permits to detect the presence of specific molecular structures, without structural identification of each peak. Moreover, some compounds cannot be distinguished only on the basis of their mass spectra (nitriles/alkenes exhibit very similar mass spectra) [2,5].

The ACF plot shows a very simple behaviour since it retains only a small number of the many possible molecular structure variations present in the original chromatogram, the most pronounced ones, i.e., those corresponding to the most abundant components or the most repeated ones. Therefore information on sample complexity can be easily extracted, in contrast with the original chromatogram very crowded of peaks where such information are hidden by peak overlapping. The ACF plot constitutes a *fingerprint* of the mixture since it is a simpler

Table 1
Molecular structures of 41 components identified in the tholin chromatogram (reported in Fig. 1) on the basis of full-scan MS spectra

Peak No.	Retention time (min)	Compound
1	3.34	Air
2	3.87	Methane
3	4.45	Carbon dioxide
4	5.38	Ethene
5	5.99	Ethane
6	6.95	Ammonia
7	7.15	Water
8	8.17	Ethanedinitrile
9	8.94	Cyanidric acid
10	9.06	1-Propene
11	9.25	Propadiene
12	10.1	Propine
13	12.3	1-Butene
14	12.5	Butadiene
15	13.1	2-Methyl-1-propane
16	13.4	Butine
17	13.5	2-Butene
18	14.3	Acetonitrile
19	16.1	2-Propenenitrile
20	16.5	2-Methylbutane
21	16.7	2-Pentene
22	17.0	1,2-Pentadiene
23	17.5	Propanenitrile
24	17.9	1-3-Cyclopentadiene
25	18.2	Propenenitrile
26	18.4	2,3-Pentadiene
27	19.2	2-Methyl-2-propanenitrile
28	19.4	Butanone
29	20.0	2-Methyl-propenenitrile
30	20.3	Formamide
31	20.5	Benzene
32	21.0	3-Butenenitrile
33	21.4	1H-Pyrrole
34	21.8	Pyrimidine
35	22.3	Acetamide
36	22.5	Pyridine
37	23.7	Toluene
38	24.1	2-Methylpyrimidine
39	25.5	4-Methylpyrimidine
40	26.9	Styrene
41	33.6	Benzonitrile

Retention time values were measured with a precision RSD≤ 0.05%.

plot than the original chromatogram still retaining all the information on the nature and relative abundances of the compounds present in the mixture. The *fingerprint* of a tholin sample can be used to compare samples synthesized in different laboratory conditions (e.g., CH_4/N_2 ratio in the initial mixture, its pressure and temperature, energy source and its intensity) in order to select the best experimental parameters to synthesize tholins most mimic of Titan's atmosphere [5,6]. Moreover, also information on quantitative composition of the samples can be obtained: in fact the height of ACF peaks is related to the number and the abundance of compounds with specific molecular structures. In particular, the value of nitriles/hydrocarbons ratio can be computed: even

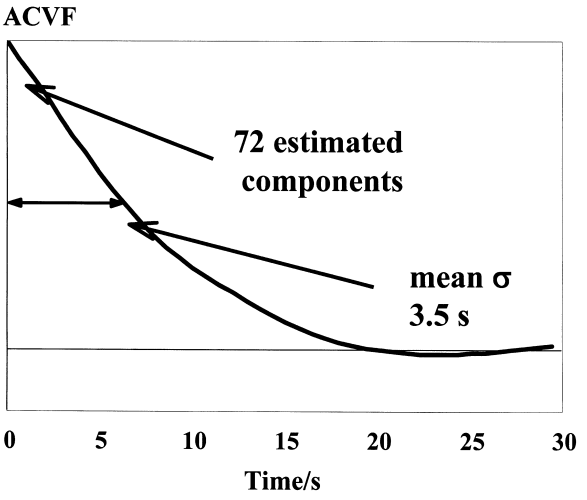

Fig. 2. Plot of the autocovariance function (ACVF) computed on the total chromatogram (reported in Fig. 1).

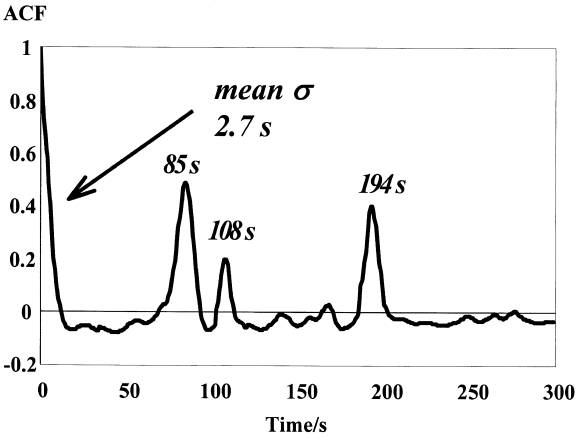

Fig. 3. Plot of the autocorrelation function (ACF) computed on the 13–30 min region of the chromatogram (reported in the detail of Fig. 1).

if these are only qualitative information, they have been found fundamental in characterizing tholin samples and can be useful to compare samples resulting from different simulation experiments [5,6].

4.2. Statistical model of peak overlapping/pulse point SMO

Values were computed for the $\overline{y_{obs}}/x_0$ couples, starting from the integrator output: various critical interdistance values x_0 were selected, corresponding to different resolution and the observed mean peak areas $\overline{y_{obs}}$ were evaluated. The ln $\overline{y_{obs}}$ quantity is plotted versus the x_0/X value, where X is the total time span of the chromatogram ($X=1801$ s): the obtained plot is reported in Fig. 4a. A linear relationship is obtained – described by the best fitting straight line – which means that the component abundances exhibit an exponential distribution, as predicted by the theoretical model [23]. The plot slope represents a statistical estimation of m, the number of single components: a value of 69 (± 3) was obtained. This result shows an excellent agreement with the value computed with the Fourier approach: the value of the statistical variable m can be estimated with an accuracy $\pm\sqrt{m}$. The good correspondence between the data, obtained by two completely independent procedures, constitutes a validation criterion of the chemometric procedures and confirms their consistency in the estimation of the number of components. Moreover, these results show an excellent agreement with literature data, obtained by using independent analysis procedures [1,3,5].

The SMO procedure was also applied to the 13–30 min portion of the chromatogram (plot reported in Fig. 4b). A close examination of the feature of the experimental points shows a step behaviour: for specific x_0/X values a steep increase of the ln $\overline{y_{obs}}$ quantity is observed. The first step is at the x_0 value of 11 s (where $x_0=4\sigma R_s$): it corresponds to the experimental limit due to the finite peak width 4σ, where resolution $R_s=1$. From this value it is possible to estimate the mean peak width, σ, obtaining a value of 2.7 s. It must be noted that this value completely agrees with that computed with the Fourier approach, that is a completely independent procedure. This is a proof of the present chemometric procedures consistency also in the estimation of the separation efficiency. On the basis of this σ value, it can be inferred that the second and third steps, at x_0 values of 16.5 and 22.8 s, correspond to resolution values of 1.5 and 2, respectively: they represent characteristic resolution values (baseline and complete separation) to identify clusters, count the peaks and calculate peak abundance distribution.

On the basis of the number of components estimated in the mixture ($m=69$) the degree of peak overlapping present in the chromatogram can be statistically evaluated using the peak overlapping statistical model [7–9], assuming an exponential distribution of single component peak interdistance. With a resolution value $R_s=0.5$, corresponding to peak maximum resolution, we can estimate that 52 peaks are observable in the chromatogram (corresponding to a separation degree $\gamma=0.83$): 43 of them are singlets (i.e., pure peaks formed by only one component), eight are doublets and one is a triplet (peaks formed by two and three components, respectively). These statistical parameters represent an accurate description of the experimental chromatogram: 50 peaks can be counted and 41 of them can be identified as pure peaks. It must underlined that statistical results describe the whole chromatogram in term of mean parameters, i.e., we can estimate the mean value of the degree of overlapping, as a general value on the whole chromatogram, without any information on which peaks are pure or not [22–24].

5. Conclusions

Two different chemometric approaches were proved to be reliable tools to characterize a GC–MS chromatogram of a tholin sample, as confirmed by the excellent agreement between the values obtained with two independent procedures. Fundamental information on the composition of the mixture (number of components, m) and on the performance of the separation system (separation efficiency, σ) can be simply estimated; moreover specific molecular structure of compounds present in the mixture can be easily singled out.

The ACF plot constitutes a *fingerprint* of the chromatogram, since it contains, in a simplified

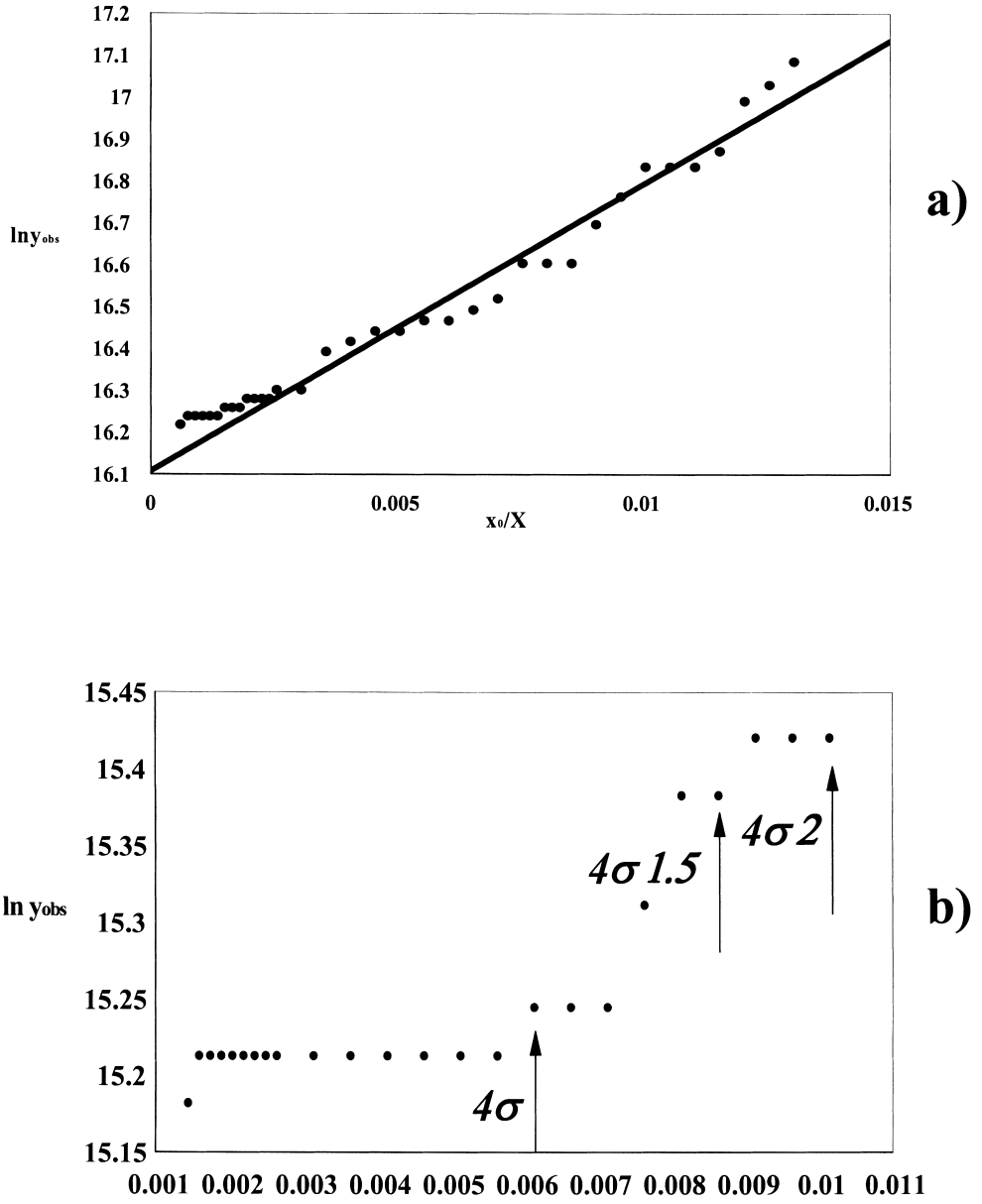

Fig. 4. Plots of ln $\overline{y_{obs}}$ vs. x_0/X values obtained by the SMO procedure. (a) Application to the total chromatogram (reported in Fig. 1). (b) Application to the 13–30 min region of the chromatogram (reported in the detail of Fig. 1).

form, the fundamental information on the mixture composition; it is useful to characterize a tholin sample to compare samples synthesized in different laboratory experimental conditions. The aim is to identify the most representative conditions to simulate the real Titan's environment, in order to understand the chemical and physical processes which take place on Titan. ACF is directly computed on the chromatographic signal itself and all the information (m, σ, retention pattern, presence of specific molecular structures) can be extracted without elaborating full-scan MS data. Therefore the ACF approach can

be applied to simpler, non hyphenated chromatographic systems, e.g., instruments used in in situ analysis in space exploration [1,3].

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