STUDIA UNIVERSITATIS BABES - BOLYAI, CHEMIA, LI, 2, 2006

Dedicated to professor Gh. Marcu at his 80th anniversary

CHEMICALLY MODIFIED BASIC ALUMINA N FOR TLC. SYNTHESIS, CHARACTERIZATION AND APPLICATIONS

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ABSTRACT. The synthesis of chemically modified basic alumina N has been performed by organosilylation reaction of hydroxylated alumina surface with trifunctional modifiers: *n*-octadecyltrichlorosilane and 3-mercaptopropyltrimethoxysilane.

These chemically modified aluminas were characterized by elemental analysis, specific surface area, FTIR and ^{13}C-CP/MAS NMR spectroscopy, mass spectrometry and thermoanalytical methods.

Chromatographic behaviour of unmodified and chemically modified aluminas were tested by the separation and identification of some dyes and benzo[a]pyrene derivatives.

Keywords: Chemically modified basic alumina N, Thin layer chromatography, FTIR spectroscopy, ^{13}C-CP/MAS NMR spectroscopy, Mass spectrometry, Thermal analysis.

The progress of chemically modified stationary phases with applicability in liquid chromatography have been permitted the use of the inorganic oxides, such as alumina, titania and zirconia, as supports due to their specific properties or as an alternative for silica gel. Alumina is a stationary phase widely used in liquid chromatography due to its special chromatographic properties and of inherent higher pH stability [1-3].

The sort of alumina frequently used in thin layer chromatography (TLC) is γ–Al_2O_3 which has on its surface three types of active centers: oxygen anions, surface hydroxyl groups and aluminium cations [4].

In order to increase the selectivity and the efficiency of the chromatographic separation, the chemical modification with a high variety of organic compounds attached to the hydroxylated surface of adsorbent is a very useful method [1,4,5].

The characterization and the applications of reversed phases based on alumina are presented in literature [2,3,6,7].

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Infrared studies on chromatographic aluminas have been mainly focused on the hydroxyl groups and Al–O–Al linkages created during the dehydration process [3]. Diffuse reflectance infrared Fourier transform (DRIFT) spectra have been used to the characterization of chemically modified aluminas [8-10]. The study of chemically modified stationary phases by mass spectrometry is not frequently met in literature [11-15].

The TG (thermogravimetry), DTG (derivative thermogravimetry) and DTA (differential thermal analysis) thermoanalytical methods are not usually used for the study of modified stationary phases. However, these methods are suitable for the characterization of chromatographic adsorbents. The shape of thermoanalytical curves gives information about the mass losses of the material and the effects that occur [7,16,17].

EXPERIMENTAL

REAGENTS:

Basic alumina N was supplied by Macherey-Nagel (Düren, Germany).

n-Octadecyltrichlorosilane, 3-mercaptopropyltrimethoxysilane, hexane and the dyes: Toluidine blue, Bromothymol blue, Sudan black B, Sudan III, were purchased from Merck (Darmstadt, Germany).

Xylene, dichloromethane, diethyl ether, acetone, benzene, ethanol, methanol, carbon tetrachloride were purchased from Chimopar Bucharest (Romania).

The benzo[a]pyrene derivatives: benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxy, benzo[a]pyrene-*trans*-9,10-dihydrodiol, benzo[a]pyrene-*trans*-7,8-dihydrodiol, benzo[a]pyrene-8-hydroxy, benzo[a]pyrene-7-hydroxy were purchased from Fluka (Switzerland).

SYNTHESIS OF CHEMICALLY MODIFIED BASIC ALUMINA N:

The chemically modified aluminas were prepared by the silylation reaction of basic alumina N with the modifiers: *n*-octadecyltrichlorosilane and 3-mercaptopropyltrimethoxysilane in the mass ratio of 2.5:1 (w/w) [7].

The obtained product was filtered and washed successively with xylene, dichloromethane and diethyl ether until the complete removal of the modifier traces.

CHARACTERIZATION OF CHEMICALLY MODIFIED BASIC ALUMINA N (Abbreviations of the studied samples are given in TABLE 1) has been performed by:

The *elemental analysis* (carbon, hydrogen and sulphur) of samples was performed by means of VARIO EL Elemental Analysis System.

The *specific surface area* of samples was determined using the BET method (krypton adsorption at the temperature of liquid nitrogen).

The *infrared spectra* of alumina samples were registered on a JASCO-610-FTIR spectrometer, using the KBr pellet technique. To improve the sensitivity of the IR method the difference and second derivative spectra were also analyzed.

^{13}C *CP/MAS NMR spectra* were recorded on a BRUKER DSX 200 spectrometer with samples of 200-300 mg in a double bearing 7 mm rotors of ZrO_2. Magic Angle Spinning (MAS) was carried out at 3500 Hz. The spectra were recorded with a proton pulse of 6.5 μs, a recycle delay of 1 s and a contact time of 1 ms.

The *mass spectra* (EI-MS) were registered on a FINIGAN MAT 311 spectrometer with double focusing in the following conditions: 70 eV electron energy, 100 μA emission current, 180°C ion source temperature. The sample has been introduced directly in the ion source and heated up to 320°C in 10^{-6} torr vacuum. The mass spectra of all samples have been continuously registered.

The *thermal analysis* was performed by means of an OD 102 Derivatograph (MOM, Hungary) in the conditions: 200 mg sample quantity, 10°C/min rate of increase of oven temperature and TG - 100 mg; DTA - 1/5; DTG - 1/5 sensitivities respectively. The measurements were conducted in air atmosphere in the temperature range of 20-1000°C.

The *chromatographic testing* was performed on 10x14 cm glass plates prepared both from unmodified and from chemically modified alumina N. The plates were coated with 0.25 mm layers by the application of an ethanolic slurry of a mixture of the stationary phase and an organic binder [6,7].

The standard solutions (0.1%) of dyes singly or as a mixture prepared in ethanol, both singly and as a mixture, were applied as 5 μL spots on the chromatographic plates by means of Brand micropipettes. Standard solutions (0.05%) of benzo[a]pyrene derivatives were prepared in benzene and spotted on the chromatographic plates in the same mode. Ascending development to a distance of 10 cm was performed at room temperature in an unsaturated Stahl chamber for all studied compounds. The dyes were developed using the dichloromethane - diethyl ether - acetone (20 + 70 + 10, v/v) mixture as mobile phase. The benzo[a]pyrene derivatives were developed using the benzene - acetone (70 + 30, v/v) mixture as mobile phase and the visualization was performed at 365 nm by a Camag lamp.

RESULTS AND DISCUSSIONS

The ideal representation of the ***synthetized chemically modified aluminas*** is shown in FIGURE 1.

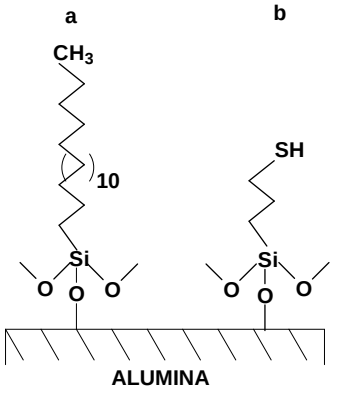

Figure 1. Chemically modified alumina: **a**, alumina-C18; **b**, alumina-SH

The obtained chemically modified aluminas were characterized by elemental analysis, specific surface area, coverage density, FTIR spectroscopy, ^{13}C CP/MAS NMR spectroscopy, mass spectrometry, thermal analysis and chromatographic behaviour by TLC separation of some dyes on unmodified and chemically modified alumina layers.

Elemental analysis data (combustion method), ***specific surface area*** (BET method) and ***coverage density*** (calculus) for unmodified and chemically modified aluminas, are presented in TABLE 1.

TABLE 1

Coverage density of unmodified and chemically modified aluminas
(% C; % H; %S; specific surface area – S_{BET}; coverage density– α)

Alumina	**Abbreviation**	**C (%)**	**H (%)**	**S (%)**	**S_{BET} (m^2/g)**	**α ($\mu mol/m^2$)**
basic alumina N	alumina	–	–	–	114.2	–
n-octadecyl basic alumina N	alumina-C18	3.22	1.66	–	89.0	1.37
3-mercaptopropyl basic alumina N	alumina-SH	2.78	0.67	1.27	96.9	7.6

The data in this table show the presence of carbon, hydrogen and sulphur and the decreasing of specific surface area of the chemically modified aluminas.

Using ***FTIR spectroscopy***, the presence of the modifier on alumina surface is well evidenced by the new bands observed in the 2800–3000 cm^{-1} range.

Due to the low concentration of the organic part of modifier on the surface, the intensity of the new bands is weak (FIGURE 2).

The bands with maximum at ~ 2923 cm^{-1} and at ~ 2853 cm^{-1} are assigned to stretching vibrations of *n*-alkyl chain: $\nu_{as}(CH_2)$ and $\nu_{sym}(CH_2)$ respectively.

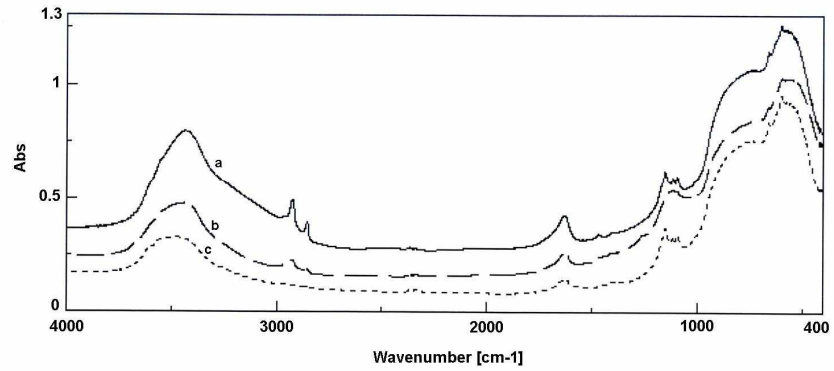

Figure 2. FTIR spectra of: **a**, alumina-C18; **b**, alumina-SH, **c**, alumina

In the difference FTIR spectrum (FIGURE 3) of unmodified and chemically modified alumina N the band observed at ~ 1468 cm^{-1} is due to the $\delta(CH_2)$ vibration. The bands observed in the range of 900-1200 cm^{-1} are assigned to ν(Al-O) and ν(Si-O) vibrations, showing the formation of Al-O-Si bridges.

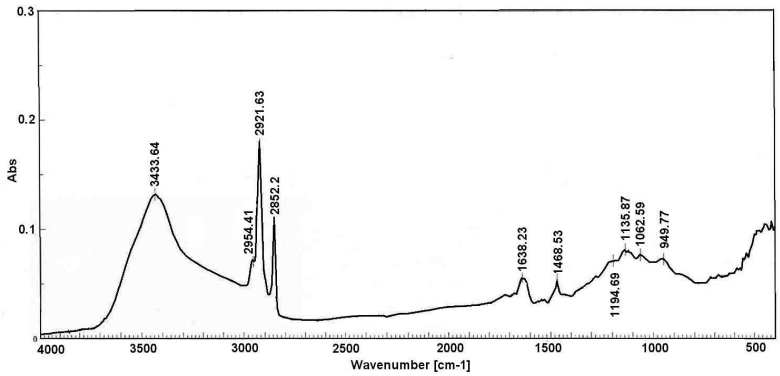

FIGURE 3. Difference FTIR spectrum of alumina-C18 and alumina

To improve the sensitivity of the infrared method, the second derivative spectra were also analysed in order to show that there are differences in the envelope of the infrared spectrum of unmodified and chemically modified alumina N (see FIGURE 4 for 3-mercaptopropyl alumina N).

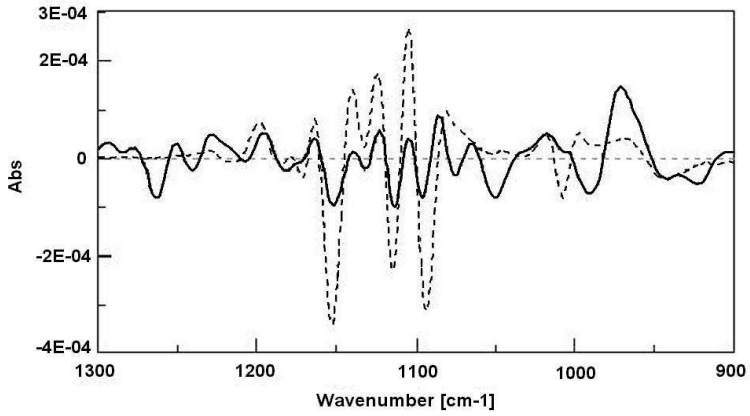

Figure 4. Second derivative spectrum of alumina-SH (–) and alumina (…)

The ***^{13}C-CP/MAS NMR spectroscopy*** was used to study the conformational properties of immobilized ligands.

The observed signals in the NMR spectrum (FIGURE 5) were assigned to the corresponding carbon atoms as it is indicated. The high field shifted signal belongs to the C-1 atom, in the neighbourhood of the silicon (12.832 ppm). The low field shifted signals are characteristic for the carbon atoms C-2 (23.07 ppm, as a shoulder) and C-3 (27.859 ppm) of the mercapto grups.

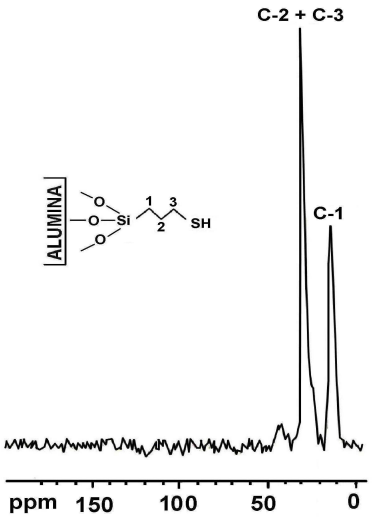

Figure 5. ^{13}C-CP/MAS NMR spectrum of alumina-SH

The comparative ***mass spectrometry*** study of unmodified and chemically modified alumina N puts in evidence the presence of the adequate chains on the modified alumina surface.

The mass spectrum (FIGURE 6) of *n*-octadecyl alumina N is dominated by the series of ions C_nH_{2n+1} (29, 43, 57, 71, …, M-15) characteristic for the long normal hydrocarbon chain [14,15]. These ions are accompanied by those of C_nH_{2n-1} (27, 41, 55, 69,…) series. The formation of both ion series can be explained based on the fragmentation rules of hydrocarbons with normal chain. The periodicity of the main ions with a mass difference of Δm 14 (CH_2) must be mentioned. The characteristic ions appear around 200°C (FIGURE 7).

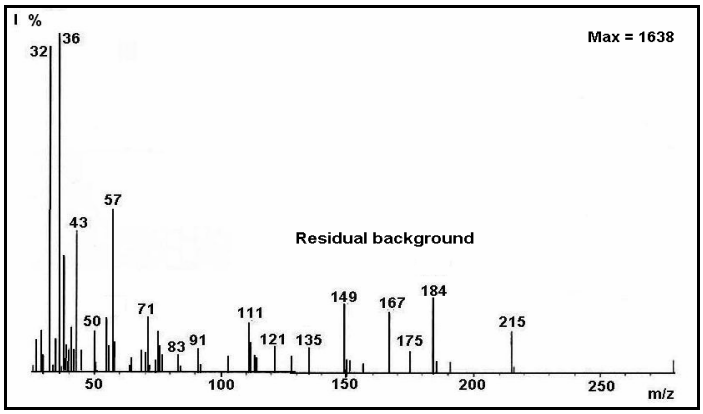

Figure 6. Mass spectrum (EI-MS) in the maximum intensity point for alumina-C18

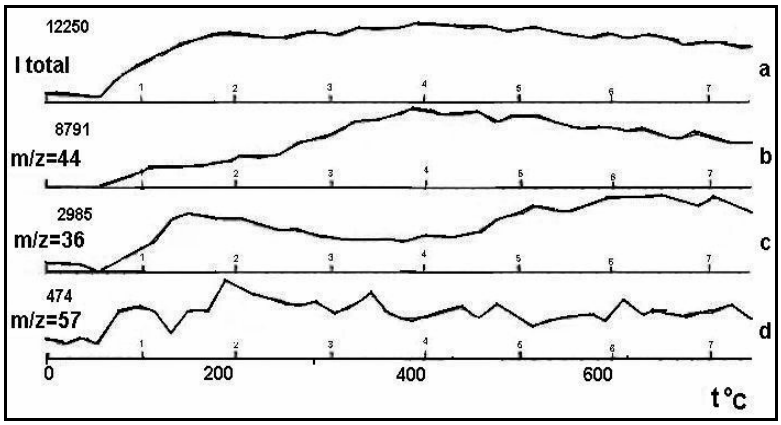

Figure 7. Intensity of characteristic ions *versus* temperature for alumina-C18: **a**) I total, **b**) m/z = 44 $[CO_2]^+$, **c**) m/z = 36 $[HCl]^+$ ion, **d**) m/z = 57 (organic part) ion

Over 100°C the characteristic ions for HCl (*m/z* 36 and 38) are visible, that confirms the fact that some initial radicals –Cl remain on the modified alumina surface.

FIGURE 8 and FIGURE 9 show the mass spectrum in the maximum intensity point and the intensity of characteristic ions *versus* temperature, for 3-mercaptopropyl alumina N, respectively.

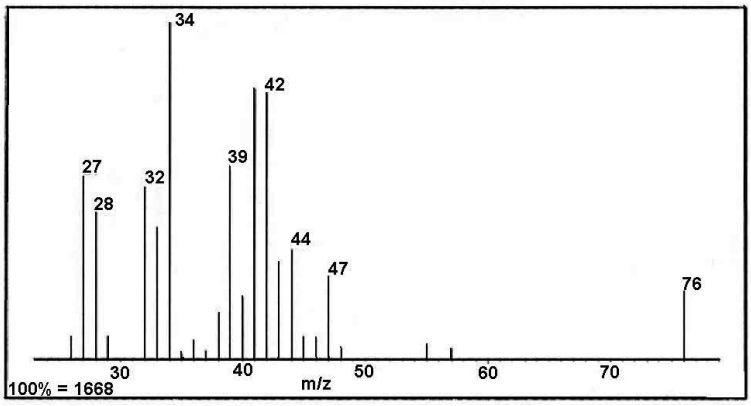

FIGURE 8. Mass spectrum in the maximum intensity point for alumina-SH

In the mass spectrum the main ions observed with: *m/z* 34, 47 and 76 correspond to SH_2, $HS\text{-}CH_2$ and $HS\text{-}CH_2\text{-}CH_2\text{-}CH_3$ structures and start around of 220°C. The characteristic ions appear around 250°C.

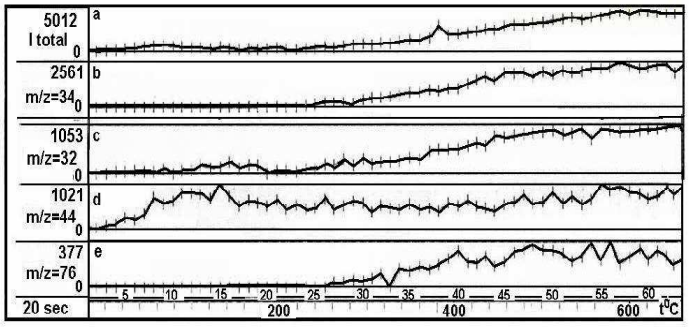

FIGURE 9. Intensity of characteristic ions *versus* temperature for alumina-SH:
a) I total, **b**) m/z = 34 $[SH_2]^+$ ion, **c**) m/z = 32 $[CH_3\text{-}OH]^+$ ion,
d) m/z = 44 $[CO_2]^+$ ion, **e**) m/z =76 $[C_3H_8S]^+$ ion

Over 180°C are visible the characteristic ions for CH_3-OH (*m/z* 32) too, confirming the remaining of some initial radicals –O–CH_3. Thus the 2,3 functionality of trifunctional modifier is explained.

The comparative study of mass spectra of unmodified aluminas *versus* chemically modified alumina, puts in evidence the modifications of alumina surface with the corresponding *n*-octadecyl and 3-mercaptopropyl chains.

The ***thermoanalytical (TG, DTG, DTA)*** method gives quantitative information about the water removed by the desorption and dehydration processes (endothermal effect) and the elimination process of organic part (exothermal effect) of the studied stationary phases and data referring to the temperature ranges where the thermal effects take place.

Thermoanalysis results for aluminas (unmodified and chemically modified) are presented in TABLE 2 and FIGURE 10.

TABLE 2

Thermoanalysis results for unmodified and chemically modified aluminas

Stationary phase	***Endothermal effect t°C***	***Δm %***	***Exothermal effects***				***ΔM %***
			Effect I, t°C	***Δm_I %***	***Effect II, t°C***	***Δm_{II} %***	
alumina	**80** (35–90)	1.0	**165, 350** (90–700)	11.5	–	–	12.5
alumina -C18	**95** (35–160)	1.8	**360** (160–480)	24.2	**560** (480–700)	5.5	31.5
alumina -SH	**80** (100–240)	8.5	**340** (240–410)	11.0	**520** (410–800)	12.0	31.5

The TG data indicate a continuous mass loss which depends on the nature and length of the chain bonded to alumina surface. The mass loss is less for unmodified alumina than for the modified material.

The DTA data show two types of thermal effects. The endothermal effect was attributed to the desorption and dehydration processes of superficial water. For the modified alumina N, the observed exothermal effects are assigned to the

removal of species from the surface and to the elimination process of the organic part (see TABLE 2).

The different thermal behaviour of unmodified and modified alumina revealed the differences among these compounds as a result of the organosilanization reaction.

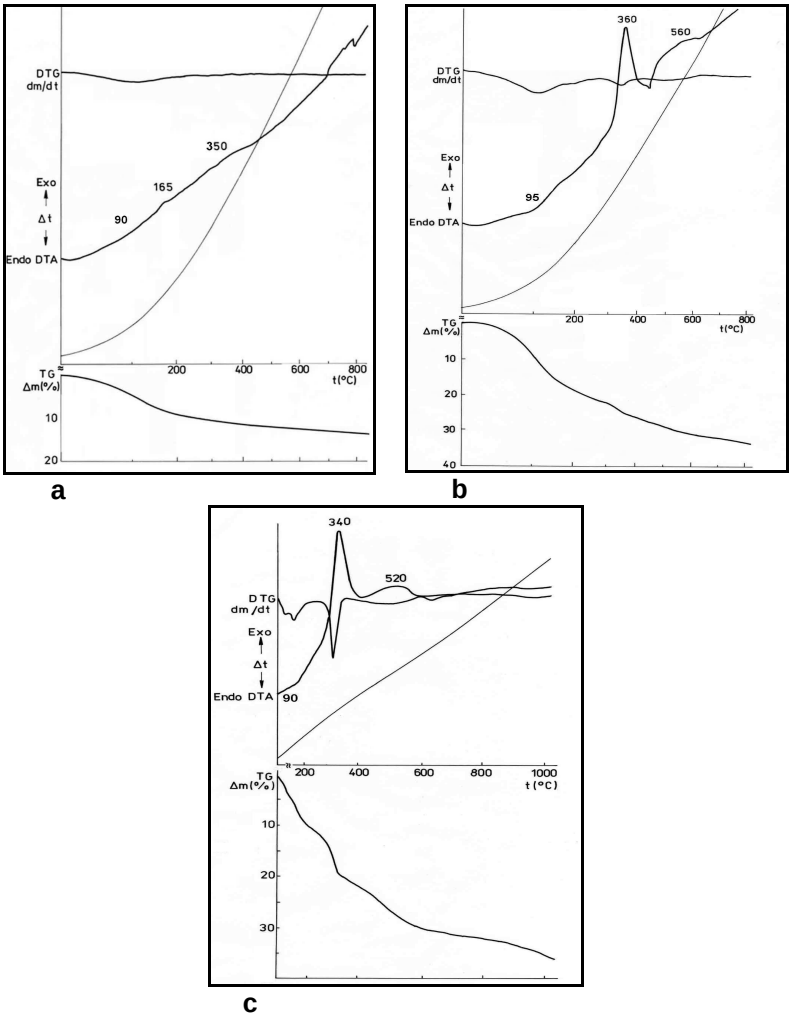

Figure 10. Thermoanalytical (TG, DTG, DTA) diagrams for:
a) alumina, **b**) alumina-C18; **c**) alumina-SH

The ***chromatographic testing*** of unmodified and chemically modified alumina N has been studied for the separation of some dyes and benzo[a]pyrene derivatives.

The parameters that define the TLC behaviour of the separated compounds on the unmodified and chemically modified aluminas [selectivity (β), efficiency (E) and resolution (R_S)] were calculated according to Equations 1-3 [18], for two neighbouring spots of studied compounds.

$$\beta = \frac{R_{f2} - R_{f1} \times R_{f2}}{R_{f1} - R_{f1} \times R_{f2}} \quad (1)$$

$$E = \frac{(\beta - 1)^2}{4\beta} \times (1 - R_{f2}) \times (1 - R_{f1}) \frac{1}{\sqrt{H_2 H_1}} \quad (2)$$

$$R_S = \frac{\Delta R_f \sqrt{Z_f - Z_0}}{2(\sqrt{R_{f1} H_1} + \sqrt{R_{f2} H_2})} \quad (3)$$

where:

$H = \frac{Z_f - Z_0}{N}$, is the theoretical plate height and $N = 16\left(R_f \frac{Z_f - Z_0}{\delta_x} \right)^2$, the theoretical plate number. R_{f1}, R_{f2}, represent the retention factors of a neighbouring pair of substances, Z_f-Z_0, the distance between origin and the mobile phase front and δ_x, the spot diameter.

The experimental average values for δ_x are 0.5 cm for unmodified alumina and 0.4 cm for chemically modified aluminas.

In FIGURE 11 are presented the chromatographic results obtained at the separation of some dyes on thin layers of unmodified and chemically modified alumina N using the dichloromethane : diethyl ether : acetone, (20 / 70 / 10, v/v) mixture as mobile phase.

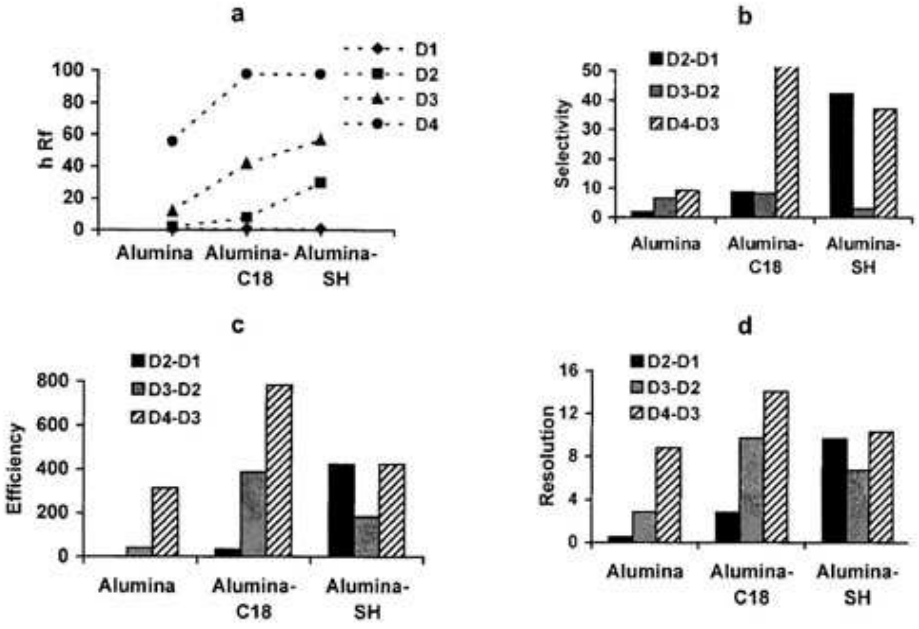

Figure 11. TLC separation of some dyes on unmodified and chemically modified basic alumina N: **a** –hR_f values **b** – Selectivity, **c** – Efficiency, **d** – Resolution
Mobile phase: dichloromethane:diethyl ether:acetone, (20 / 70 / 10, *v/v*);
Dyes: Toluidine Blue (D1), Bromothymol Blue (D2), Sudan Black (D3), Sudan III (D4)

The chromatographic results obtained at the separation of benzo[a]pyrene derivatives on unmodified and chemically modified aluminas are presented in FIGURE 12.

The results show that the values of the chromatographic parameters depend on the retention factors of two neighbouring compounds, the migration distance of mobile phase, the diameter of spots and the type of mobile and stationary phases, respectively.

Analysing the results presented in FIGURE 12 we can conclude:

- for $\boldsymbol{R_f}$ values from the 0.2-0.8 range, the selectivity has a small variation;
- the separation degree of two neighbouring compounds is given by the value of resolution;
- the efficiency of separation of two neighbouring compounds depends on the selectivity, $\boldsymbol{R_f}$ values and spot diameter.

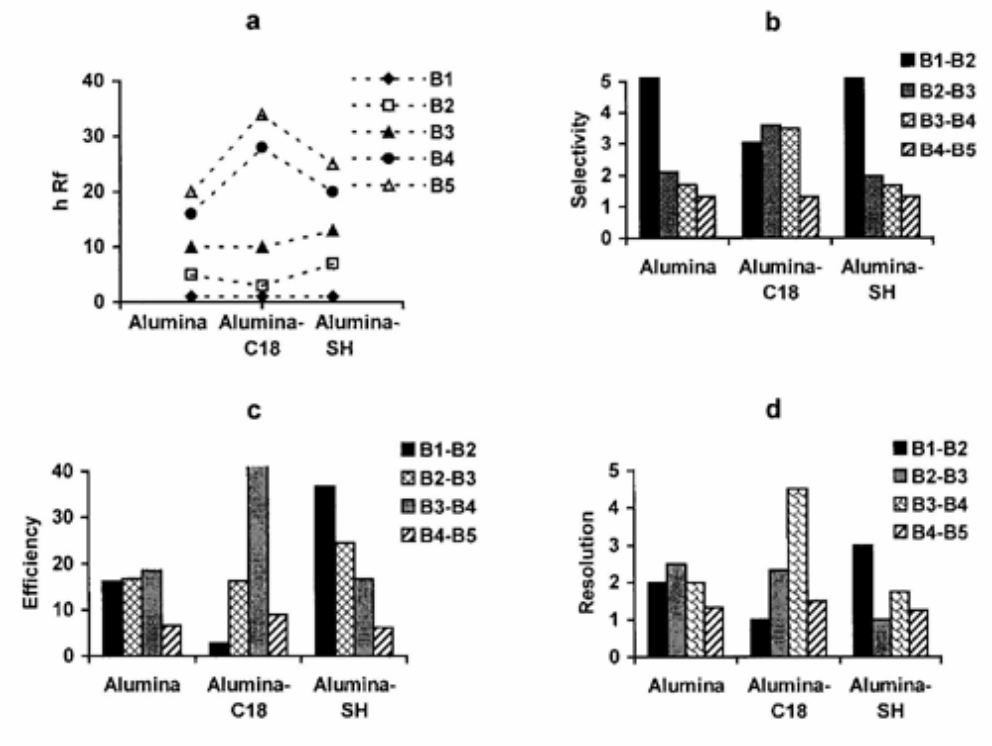

Figure 12. TLC separation of benzo[a]pyrene derivatives on unmodified and chemically modified alumina N: **a** – hR_f values; **b** – Selectivity; **c** – Efficiency; **d** – Resolution.
Mobile phase: benzene:acetone (70 / 30, *v/v*);
Benzo[a]pyrenes: Benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxy (*syn*) (B1), Benzo[a]pyrene-*trans*-9,10-dihydrodiol (B2), Benzo[a]pyrene-*trans*-7,8-dihydrodiol (B3), Benzo[a]pyrene-8-hydroxy (B4), Benzo[a]pyrene-7-hydroxy (B5

The best values of the chromatographic parameters at the separation of some dyes and benzo[a]pyrenes derivatives have been obtained on *n*-octadecyl alumina N thin layers.

The chromatographic results demonstrated new retention properties for the chemically modified aluminas and the possibility to use them at different chromatographic applications.

CONCLUSIONS

Chemically modified alumina N samples have been obtained by the silylation reaction of basic alumina N with trifunctional modifiers, namely *n*-octadecyltrichlorosilane and 3-mercaptopropyltrimethoxysilane.

Elemental analysis and FTIR spectra of modified alumina show the presence of organic modifier on the alumina surface. An important change of specific surface area is obtained.

The comparative mass spectrometry and ^{13}C CP/MAS NMR spectroscopy study of unmodified and chemically modified alumina put in evidence the presence of the adequate chains on the modified alumina surface.

Thermoanalytical diagrams permitt to find out the type of thermal effects and the temperature ranges where they take place, as well as the corresponding mass loss values.

The chromatographic behaviour of chemically modified alumina proves their new retention properties at the separation of some dyes and benzo[a]pyrene derivatives.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS GRANT No. 318/2000-2002.

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