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Use of near infrared emission spectroscopy in the study of supporting materials and stationary phases for liquid chromatography

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Abstract

Near infrared emission spectroscopy (NIRES) has been investigated in the study of different materials employed in liquid chromatography. The samples were heated in a nitrogen atmosphere and the emission spectra were obtained using a lab-made NIRES instrument. Through principal component analysis (PCA) using the raw emission spectra, it was possible to distinguish different materials according to their physical and/or chemical characteristics. Linear relationships between emissivity spectra and the contents of the coating material or the specific surface areas was observed for stationary phases or bare silicas, respectively. Furthermore, the thermal stability of stationary phases could be followed in real time.

Keywords: Near infrared emission spectroscopy; Stationary phase characterization; Liquid chromatography

1. Introduction

The characterization of new materials and the unambiguous identification of commercial materials employed as stationary phases in chromatography is a subject of great interest in the development and practical use of chromatographic techniques.

Some well-established analytical techniques are usually employed for the characterization and identification of such materials. Among the most employed are: elemental analysis [1–9], reflectance or transmittance Fourier transform infrared spectroscopy (FTIR) [1–12], thermal techniques including thermogravimetry and differential scanning calorimetry [1,6,9,11,12] and ^{13}C and ^{29}Si solid-state nuclear magnetic resonance (NMR) [3,5–9,11,12]. Other techniques employed with lower frequency include: atomic force microscopy (AFM), scanning electron microscopy [6,10], and Raman spectroscopy [13,14]. These techniques are complementary, giving information about several relevant aspects related to the structural and physico-chemical characteristics of the materials used as stationary phases.

In infrared emission spectroscopy, a sample is thermally excited to higher vibrational energy levels and the radiant relaxation process is monitored by a spectrometer. A molecular emission spectrum is recorded whose emission maxima agree with the absorption maxima of the conventional absorbance spectra of the same samples in terms of wavelength position and relative intensities. Although near infrared spectroscopy (NIR) is widely applied to real-life analytical problems, most of the works dealing with infrared emission spectroscopy still use the mid-infrared spectral region. Mid-infrared emission spectroscopy (MIRES) requires lower excitation temperatures to obtain measurable emission signals, but near infrared emission spectroscopy (NIRES) can be used with greater sample thicknesses, compensating its lower emission intensities.

NIRES was recently implemented, to advantage, using an Acousto-Optical Tunable Filter-based instrument (NIRES-AOTF) and has been investigated as an alternative technique for characterization of solid materials [15]. A small amount of the sample (1–3 mg) is heated at a selected temperature (above 100 °C) and its emission spectrum is registered in the range from 1500 to 3000 nm. The spectral features of the near infrared region can reflect the structure of the material as well its physico-chemical characteristics. The main contributions to these features come from thermally excited vibrational states

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(for instance, overtones and/or combinations) of chemical bonds between the hydrogen atom and any other heavier element such as oxygen and carbon in the materials employed in liquid chromatography [16].

This work investigated the use of NIRES-AOTF in the study of common materials employed as supporting materials and stationary phases for liquid chromatography. Qualitative and quantitative results are presented that allow predicting the use of NIRES-AOTF for identification/classification and characterization of such materials.

2. Experimental

2.1. Instrumentation

The NIRES instrument is based on an AOTF made with a TeO_2 crystal, manufactured by Brimrose (TEAF_1.5–3.0) to operate in the spectral region from 1500 to 3000 nm with nominal spectral resolution varying from 9 to 37 nm as the selected wavelength increases in its useful range. The AOTF was driven by a radio frequency (rf) signal generated by a digital synthesizer (Analog Devices, AD 9852), assembled to be controlled by a parallel interface (ICP-DAS A8111) placed into a micro-computer running a customized software written in Visual Basic 5.0. Under computer control, the synthesizer board generates a rf signal in the range of 68–34 MHz, necessary to select the wavelength emitted by the sample in the 1500–3000 nm range, respectively. The rf signal is modulated with a TTL level signal at 167 Hz and amplified by a rf amplifier (RF Gain, BBM2C4AJT, 8 W, 10–1000 MHz) to produce a 3.0 W signal applied to the AOTF. Therefore, the radiation of selected wavelengths is also modulated at this frequency. The detector is based on a thermoelectrically cooled (−10 °C) PbS element (Ealing Electro-Optics 043/035) aligned at 90° in relation to the plane of the output window of the AOTF, allowing the measurement of both diffracted beams superimposed on the non-diffracted beam with the modulation. A lock-in amplifier (Stanford Research SR830 DSP) was employed for synchronous acquisition of the modulated signal. The analogue output of the amplifier was connected to the parallel interface, which was responsible for the conversion of the analogue signal to the digital domain with a 12 bit resolution. The sample cell was a rod made of aluminum with 1.4 cm diameter and 0.6 cm height, where a shallow circular groove was machined with 0.6 cm diameter and 0.3 cm depth. The cell is attached to the top of an aluminum cylinder of the same diameter that contains a 60 W electrical heater. The average power applied to the heater was controlled by home made electronic circuitry based on the duty cycle of an oscillator driven ac switch. The entire heating system was contained in an acrylic tube of 3.3 cm inner diameter with 0.3 cm wall thickness, which provides thermal isolation and the possibility of nitrogen insertion at a low flow rate for free oxygen applications. The temperature of the sample was monitored by a calibrated thermocouple inserted in a small hole drilled in the body of the cell, at the point nearest to the sample. A digital meter was set to monitor the thermocouple signal, which was connected through a RS-232 serial interface to the system-controlling computer. The instrument was described in greater detail in a previous work [15].

2.2. Materials investigated

The materials used are described in Table 1. The materials were selected to include commercially available products as well as some new materials recently investigated for use as stationary phases in high performance liquid chromatography [2,4,6].

2.3. Spectral data acquisition and data treatment

For each material studied, 2.0 mg were weighed, transferred to the sample cell and heated in a nitrogen atmosphere (to simulate the free oxygen environment inside the chromatographic columns). No pre-treatment was employed and the samples was taken as supplied. For a preliminary classification, raw emission spectra, acquired at 180 °C as averages of 10 scans (1 min for each scan) with a nominal resolution of 5 nm, were smoothed by a moving average filter (3 point window), baseline corrected, normalized by the maximum value of intensity, mean centered and submitted (region from 2100 to 2700 nm) to principal component analysis (PCA) using a chemometrics software (Unscrambler 9.1, CAMO, Norway). Two replicates of each sample were employed to evaluate the repeatability of the spectral data. The PCA performs a dimensional reduction of the multivariate data (121 dimensions) and each spectrum produces score values in few dimensions, called principal components, which explain as much data variability as possible [17]. Therefore, the scores graph in the first two or three principal components allows a visualization of differences and similarities between samples. For thermal stability studies, after 10 scans at 180 °C, the temperature was elevated to 220 or 250 °C and the emission signal was monitored at 2290 nm (a wavelength whose signal was related to the coating materials) for 1800 s (time intervals of 30 s). For a better relationship between emission intensities and the concentration of the emitting species or sample thickness, raw emission signals could be converted to the emissivity scale (ε) [18], using the equation:

$$\varepsilon = -\log\left(1 - \frac{\mathrm{SS}}{\mathrm{BS}}\right)$$

where SS is the raw emission signal of the sample and BS is the raw emission signal of a blackbody at a given wavelength, taken at same temperature. The experimental blackbody was an aluminum rod matched in terms of size with the sample cell, but with its shallow circular groove blackened with matte black spray paint. For comparison purposes, NIR absorbance spectra of four bare silica samples (Brimrose Luminar Spectrometer equipped with Free Space Luminar 2030) and thermograms of two stationary phase samples (TA Instruments TGA 2050) were obtained. The absorbance spectra were obtained, by means of reflectance measurements, as averages of 50 scans with a nominal resolution of 2 nm and the thermograms were obtained in argonium atmosphere with a temperature scan rate of 10 °C min^{-1} within the range of 23–900 °C.

Table 1
Description of the materials investigated in the work

Sample type	Supplier	Particle diameter (μm)	Pore diameter (nm)	Form	Coating material	Commercial name
Bare silica	SAI	32–63	6	Irregular	–	–
	Ultrachem	35–70	6	Irregular	–	–
	SAI	63–200	6	Irregular	–	–
	Alltech	10	15	Irregular	–	Davisil 150-10
	Merck	5	6	Irregular	–	Lichrosorb 60-5
	Merck	7	6	Irregular	–	Lichrosorb 60-7
	Merck	10	10	Irregular	–	Lichrosorb 100-10
	Nomura	7	3	Spherical	–	Develosil 30-7
	Macharey-Nagel	7	5	Spherical	–	Nucleosil 50-7
	Varian	5	10	Spherical	–	Rainin 100-5
	Akzo Nobel	5	10	Spherical	–	Kromasil 100-5
	Akzo Nobel	16	30	Spherical	–	Kromasil 300-16
Stationary phase	Akzo Nobel	10	–	Spherical	Octadecylsilane[a]	Kromasil C-18
	Shandon	3	–	Spherical	Dimethyloctylsilane[a]	Hypersil MOS-2
	Shandon	5	–	Spherical	Octylsilane[a]	Hypersil WP-300
	Shandon	10	–	Spherical	Octadecylsilane[a]	Hypersil ODS
	Merck	10	–	Irregular	Octadecylsilane[a]	Lichrosorb RP-18
	Merck	10	–	Irregular	Octylsilane[a]	Lichrosorb RP-8
	Lab-Made	10	–	Irregular	PDMS[b]	Davisil + PDMS
	Lab-Made	10	–	Irregular	PMOS[c]	Davisil + PMOS

[a] Chemically bonded.
[b] Sorbed phase with 20, 25, 30 or 40% PDMS.
[c] Immobilized phase at 110, 120 or 130 kGγ.

3. Results and discussion

Fig. 1a shows the complete smoothed, baseline corrected and normalized raw emission spectra set obtained for the materials described in Section 2. Based on this data set it is possible to select the spectral range from 2100 to 2700 nm as the region containing most of the spectral variability and, therefore, most of the information about the different materials studied. In consequence, the remaining discussion will be focused in this spectral region.

3.1. Sample classification

For a preliminary classification seeking the observation of differences and similarities between the samples, the pre-treated raw emission intensities from 2100 to 2700 nm were submitted to PCA. Fig. 1b shows the scores distribution of the samples in the first two principal components, which explain about 97% of the spectral variability found in the spectra set of the samples. The analysis of the distribution of the scores shows that the raw emission spectra (without conversion to a proper emission intensity scale, as emissivity) can be used to classify the different materials employed as supporting materials and stationary phases in liquid chromatography. The first PC practically splits the samples into four groups: bare silicas, stationary phases containing poly(dimethylsiloxane) (PDMS), stationary phases containing poly(methyloctylsiloxane) (PMOS) immobilized by γ-radiation, and stationary phases containing other bonded alkyl groups. The bare silicas were grouped apart because their spectra presented only one band between 2100 and 2500 nm whereas spectra of stationary phases presented several bands within this spectral range. The second PC further improves the classification by differentiating among stationary phases containing different quantities of PDMS, stationary phases containing PMOS immobilized with different doses of γ-radiation, stationary phases from different suppliers (Kromasil, Lichrosorb and Hypersil) and stationary phases coming from a same supplier but having different coating materials (Hypersil having bonded octadecylsilane, dimethyloctylsilane or octylsilane).

Although the raw emission spectrum can supply the necessary information for classification of the different materials, it does not allow the observation of the spectral features and details responsible for such differentiation. On the other hand, it has been demonstrated that the intensities expressed as emissivity present a linear relationship with the sample thickness and the concentration of emitting species and the profile of the emissivity spectrum is very similar to that of the absorbance spectrum in terms of peak position and relative intensities [15]. In fact, for some samples it was possible to establish a relationship between spectral intensities in emissivity and quantitative parameters. Therefore, the following discussion will be focused on analysis of emissivity data.

3.2. Concentration of coating material

In the development of new stationary phases for liquid chromatography, sometimes different amounts of coating material bonded to silica particles are used with the purpose of verifying the optimal quantity of coating material in relation to the column performance. So, it is also important to verify if the desired quantity of the coating material in the final stationary phase develops. This information can be accessed by the emissivity spectra. Fig. 2a shows the emissivity spectra obtained for bare Davisil silica and the same silica covered with different amounts

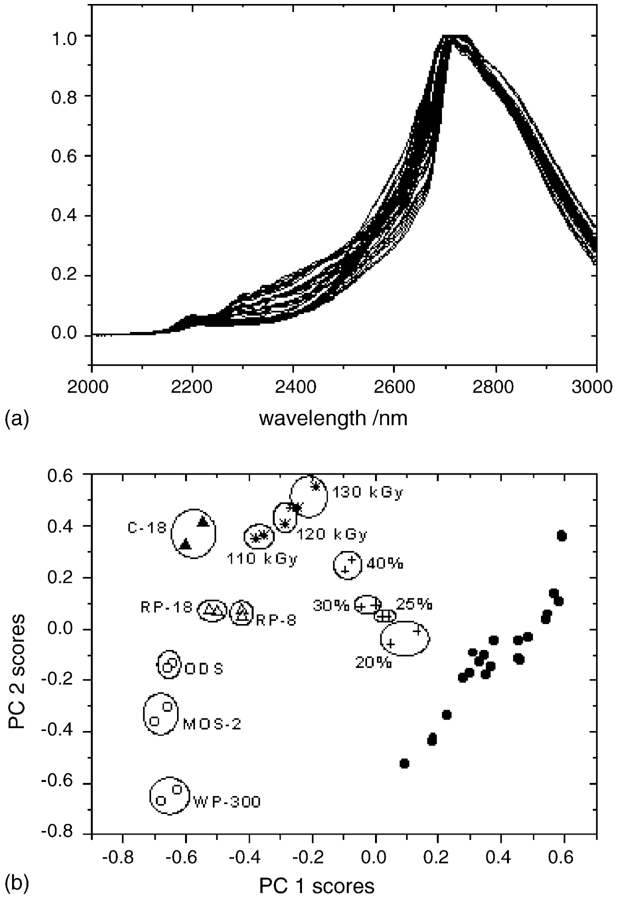

Fig. 1. (a) Raw emission spectra of the materials utilized in this work; (b) score distribution of the samples for the first two principal components after PCA. Solid circles, bare silicas; open circles, Hypersil stationary phases; solid triangles, Kromasil stationary phases; open triangles, Lichrosorb stationary phases; crosses, stationary phases containing PDMS; asterisks, stationary phases containing PMOS immobilized with γ-radiation.

of PDMS. It is possible to observe the emission bands associated with the methyl groups, related to the coating material, attributed to combinations of C–H group vibrations. Keeping the mass of the sample constant, the emission spectra reflect the quantity of PDMS covering the silica support. A linear relationship (slope of 0.0037, intercept of −0.0446 and correlation coefficient of 0.994) was found between the peak height at 2285 nm and the PDMS content. The peak heights at 2360 or 2460 nm can also be used, resulting in linear relationships with the PDMS content on the stationary phases.

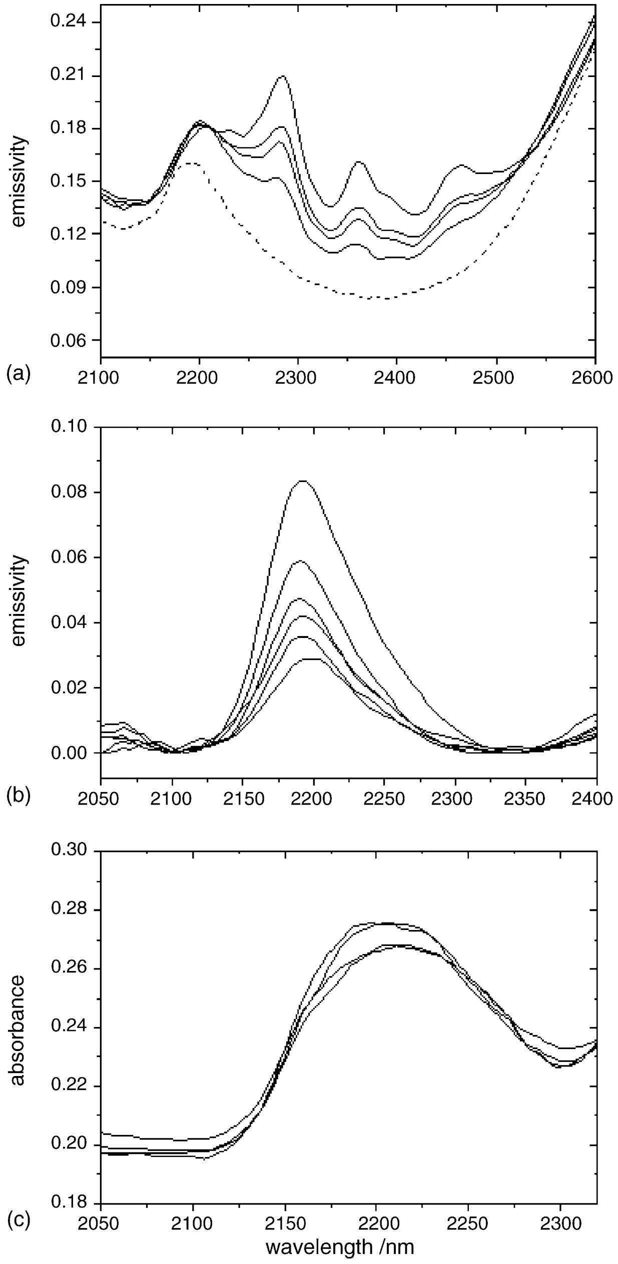

Fig. 2. (a) Spectra obtained for bare Davisil (dashed line) and the same silica covered with, from bottom to top, 20, 25, 30 and 40% of PDMS; (b) spectra showing the effect of the specific surface area (SSA) on the emissivity for bare silicas, from bottom to top: Kromasil 300-16 (SSA = 87 m^2/g), Rainin 100-5 (SSA = 190 m^2/g), Davisil 150-10 (SSA = 237 m^2/g), Kromasil 100-5 (SSA = 313 m^2/g), Nucleosil 50-7 (SSA = 420 m^2/g), and Develosil 30-7 (SSA = 653 m^2/g); (c) absorbtion spectra for Rainin 100-5, Davisil 150-10, Nucleosil 50-7 and Develosil 30-7.

3.3. Specific surface area

For bare silica samples, only one emission band was observed in the region up to 2400 nm. This band, at 2200 nm, is described as a combination of Si–OH bending and O–H stretching [19]. As the OH groups exist only on the surface of the bare silica particles, the number of OH groups is related to physical parameters such as the specific surface area. In this way, the intensity of the band at 2200 nm reflects the total number of OH groups and can be associated to this physical parameter. The specific surface area is a very important parameter in the development of new stationary phases because it is related to the capacity of bare silica particle to bind a coating material, since the coating material can only be bonded to OH groups in the surface of silica particle. Fig. 2b shows the emissivity spectra for six bare silica samples with known specific surface areas, determined through the BET nitrogen adsorption technique [20]. The spectra were submitted to a linear baseline correction between 2100 and 2350 nm. A linear relationship (slope of $9.56 \times 10^{-5} \pm 2.9 \times 10^{-6}$, intercept of 0.0186 ± 0.0011 and correlation coefficient of 0.998) between the specific surface area and the peak height at 2200 nm was observed. The explanation for this comes from the fact that, for a defined mass of sample, the higher the total surface area, the higher the total number of OH groups. The relationship obtained can be used to estimate specific surface areas of similar silica samples, with the advantages of lower sample consumption and more rapid analysis time in comparison with the nitrogen adsorption technique. For comparison purposes, the absorbance spectra of four bare silica samples (from the six samples) were obtained using a commercial NIR spectrometer based on reflectance measurements. The samples were previously dried in a furnace and the absorbance spectra obtained are shown in Fig. 2c. As can be observed, there is not any correlation between the absorption band at 2200 nm and the specific surface areas of the samples, demonstrating the advantage of NIRES over NIR absorption methods for this application. Besides, no pre-treatment of the samples was necessary in NIRES and the heating of the samples along the emission measurements guarantee a humidity-free environment in the surface of silica particles.

3.4. Thermal stability

As NIRES requires the thermal excitation of the samples, this technique can also be used to study the thermal stability of different stationary phases. To demonstrate this application, samples of Lichrosorb stationary phases containing different coating materials were used for comparison between its thermal stabilities. Fig. 3a shows consecutive spectra (300 s for each spectrum) for the Lichrosorb having bonded octylsilane sample heated at 250 °C. The decrease of emission intensities between 2200 and 2500 nm (the spectra approximating those for bare silicas) indicates the thermal degradation of the coating material at this temperature. By monitoring the emission intensities at 2290 nm (wavelength of an emission band whose intensity decreases in Fig. 3a) with heating time, it is possible to determine the relative degradation rate of the stationary phase sample. This was done for the Lichrosorb sample cited above and also for a Lichrosorb having bonded octadecylsilane, using the same emission band at 2290 nm, for heating temperatures of 220 and 250 °C, as shown in Fig. 3b and c, respectively. For each sample, it is possible to observe a faster decrease of the emission intensity and, consequently, a faster degradation rate at the higher temperature, which demonstrates that these intensity decreases is related to thermal degradations. For each temperature, it is possible to observe that the emission intensity of the sample containing octadecylsilane presents a faster intensity decrease and, consequently, a faster degradation rate, indicating lower thermal stability. This observation was confirmed by thermogravimetric

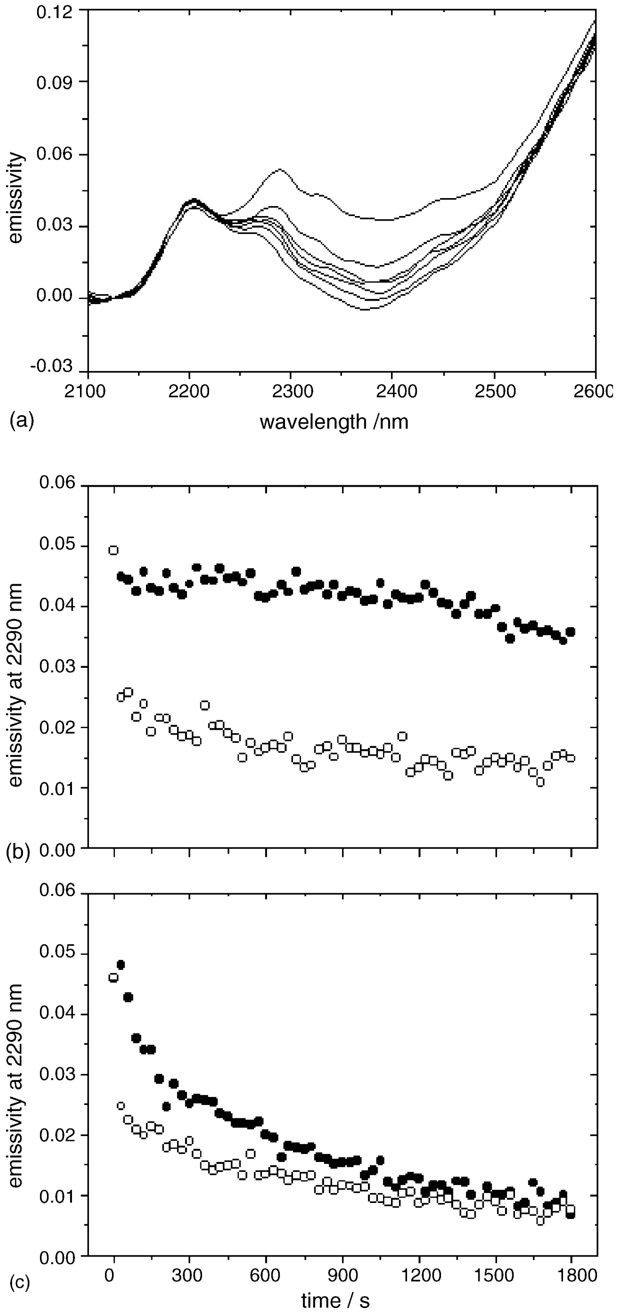

Fig. 3. (a) Consecutive emissivity spectra, from top to bottom, of Lichrosorb having bonded octylsilane showing thermal degradation. (b), (c) Emissivity at 2290 nm decreases with time of heating at 220 and 250 °C, respectively. Solid circles, Lichrosorb having bonded octylsilane; open circles, Lichrosorb having bonded octadecylsilane.

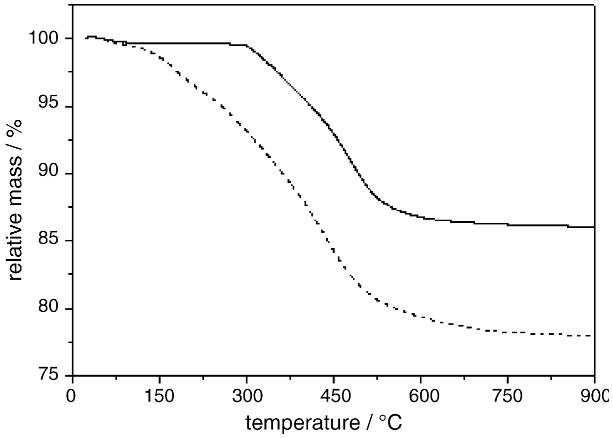

Fig. 4. Thermograms of Lichrosorb stationary phases having bonded octylsilane (solid line) and octadecylsilane (dashed line).

analysis of these samples, as shown in Fig. 4. Although, in liquid chromatography, the chemical stability (in the presence of the solvent used as mobile phase) is more important than the thermal stability (without solvent) and chromatographic columns in that technique are not commonly heated at the temperatures used in this thermal stability study, it demonstrates an interesting possibility of NIRES application, which can be useful in a future work, for example, to evaluate the thermal stability of stationary phases used in gas chromatography. However, the lower thermal stability of stationary phases having bonded octadecylsilane in relation to stationary phases having octylsilane at high temperatures was already observed in liquid chromatography in a work using subcritical water as mobile phase [21].

4. Conclusion

Although a complete attribution of the spectral features achieved by NIRES-AOTF is more difficult than it is for mid-infrared spectroscopy, due the more complex nature of the NIR spectral region, the emissivity spectrum and even the raw emission spectrum (which precludes the blackbody reference spectra) contains, as demonstrated by the results shown in this work, enough information to be proposed as an alternative technique for the characterization of different materials employed as supporting materials and stationary phases in liquid chromatography.

The NIRES information can be used to obtain both chemical and physical characteristics of the different materials studied. Its has been shown that the information present in the raw emission spectra is sufficient for a preliminary classification of the samples, allowing the characterization of different materials in an unambiguous way. Some chemical characteristics of the coating materials and of the bare silica surfaces can be promptly identified by using the spectral features in the region from 2100 to 2600 nm. This region is filled with information coming from the combination bands of the C–H, O–H and Si–OH vibrational modes. Therefore, the emissivity spectra in this region can be related to the quantity of the coating material in stationary phases and to the total number of OH groups on bare silicas. This last feature, making use of the intensity of the emission peak at 2200 nm, can be related to the specific surface area, one of the most important physical parameters of bare silicas. Furthermore, NIRES-AOTF can be used to follow the thermal behavior of the material being evaluated as a stationary phase and indicates its potential stability on the short or long term. This facility is valuable for the development of new stationary phases for gas chromatography or high temperature liquid chromatography.

One of the most attractive characteristics of NIRES is the low sample consumption (about 2 mg per analysis) and the absence of any sample pre-treatment. This is very important when a new stationary phase is being developed and is available only in small quantities. Besides, a NIRES-AOTF instrument can be easily implemented and possesses lower instrumental costs in relation to other techniques commonly employed for characterization of supporting materials and stationary phases for liquid chromatography, such as FTIR, BET, NMR and AFM, for example. For specific surface area determination, advantages of NIRES over NIR absorption spectroscopy by reflectance and BET were demonstrated, as the lower analysis time in addition to the lower sample consumption. For thermal stability studies, NIRES can provide information similar to that obtained with thermogravimetric analysis.

The results shown in this work permit proposing a possible complementary role of NIRES-AOTF for characterizing and classifying new and/or commercial materials employed as supporting materials and stationary phases for liquid chromatography.

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