

**RELATIONSHIP BETWEEN RAMAN SCATTERING
INTENSITY IN THE AROMATIC REGION
AND THE THERMALLY INDUCED INCREASE
IN THE UV ABSORPTION
OF CHEMICALLY BONDED STATIONARY PHASES**

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SUMMARY

In the initial papers reporting the thermal instability of chemically bonded stationary phases on irradiation with a high-power neodymium laser, Raman scattering intensity in the region 2285 to 1130 cm^{-1} was found to depend on the density of coverage of the silica matrix with alkyl ligands. Further research focused on the dependence of the growth of UV absorption in this region, on heating of the stationary phase, on the density of coverage of the silica matrix with alkyl ligands capable of aromatization. For thermal modification of the RP-2 stationary phase impregnated with paraffin oil a quantitative dependence was found between the growth of absorption in this UV region and the paraffin oil-content of the chromatographic bed.

In this study the relationship between the thermally induced increase in UV absorption of RP-8 and RP-18-type stationary phases and Raman-scattering intensity in the aromatic region when the stationary phases are irradiated with a high-power neodymium laser is discussed. The results obtained confirm the assumption of a linear relationship between the growth of UV absorption in this region by the stationary phase as a result of its thermal modification and the density of coverage of the silica matrix with alkyl (octyl or octadecyl) ligands capable of aromatization.

INTRODUCTION

Spectroscopic study of chemically bonded stationary phases provides abundant and diverse information about their composition and structure. Raman spectroscopy supplies much valuable data about the surface of the modified silica. By use of these data it is, e.g., possible to study the effect of temperature, mobile phase components, and the density of coverage of the silica matrix with alkyl ligands on the conformational order of the stationary phase [1–4]. For stationary phases based on silica with chemically bonded alkyl groups, conformational order plays a very important role in the mechanism of retention, which consequently determines the efficiency and selectivity of a chromatographic system [5–8]. Chemical data referring to the surface of the modified silica and, in particular, to the structure and conformation of the chemically bonded alkyl groups, can also be obtained by many other methods, for example nuclear magnetic resonance spectroscopy (NMR) [9–15] or Fourier transform-infrared (FTIR) spectroscopy [16–24]. The data obtained might be used for optimization of stationary phase synthesis, aimed at improvement of its chromatographic efficiency and/or the reproducibility of the properties of consecutive batches of the stationary phase. These two targets are important when using these products both analytically (in striving toward more efficient separation and reproducibility) and preparatively (for which optimum system capacity and repeatability are in great demand).

The still growing popularity of chromatographic techniques for solving analytical problems has recently been emphasized by a tendency to standardize the chromatographic materials used, mainly because of the desire to obtain reliable analytical results and to establish repeatable analytical procedures. Currently, however, practical normalization of chromatographic stationary phases, especially for reversed-phase liquid chromatography, seems rather difficult (or even hardly possible). The difficulty originates mainly from the complex nature of these stationary phases (particularly the so-called ‘surface phase’) and is related to the synthetic methods used and a variety of physicochemical factors. Thus determination of the chromatographic properties of impregnated or chemically modified silica gel, on the basis of the preparation procedure, can prove quite difficult. For chemically modified silica, the quality and quantity of the chemically bonded groups per unit area of the adsorbent surface, and the quantity and the kind of polar groups (e.g. free silanols) [25,26] seem to be of significant importance. The search for novel methods of assessment of stationary pha-

ses, to gather more information on structural features affecting retention mechanisms, seems fully justified. A new method of comparison of the density of coverage of the silica gel matrix with octyl, octadecyl, 3-cyanopropyl, and diol ligands on the basis of Raman spectroscopy and, more specifically, by use of an intense and broad Raman band of obscure origin, has been introduced elsewhere [27–30]. Discovering the origin of this finely structured, intense, and broad band in the frequency range from ca 2285 to 1130 cm^{-1} proved a real challenge. Systematic study of the behaviour of the chemically bonded stationary phases of interest (RP-8, RP-18, CN, diol, and RP-2 impregnated with paraffin oil) at elevated temperatures, employing differential scanning calorimetry (DSC), UV and IR spectroscopy, and modified acquisition of Raman spectra, is described elsewhere [31]. To confirm the formation of aromatic products in chemically bonded stationary phases heated at 165°C, the extracts obtained from washing these treated adsorbents were further analyzed by HPLC–DAD [32].

The objective of this study was to determine the quantitative relationship between the amount of aliphatic groups in TLC-type adsorbents (causing an increase of the broad and intense Raman band) and UV spectral changes occurring as a consequence of thermal modification of these groups.

EXPERIMENTAL

The TLC-Type Adsorbents

In our study we used commercial glass TLC plates coated with silica, silanized silica, octyl silica, and octadecyl silica adsorbents (Table I), all manufactured by Merck (Darmstadt, Germany).

Raman Spectrometry

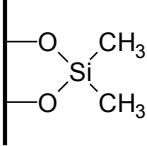
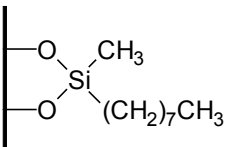
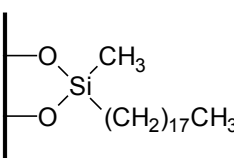
Raman spectra were acquired with a Nicolet (USA) FT Magna-IR 860 spectrophotometer using the appliance (FT-Raman module) and the Z-stage set specially devised for flat samples, which enabled acquisition of spectra directly from the plates. The other characteristics of the Raman spectroscopic equipment and the working conditions were: InGaAs detector; CaF_2 beam splitter; and laser source power 2.5 W.

Thermal Modification

Stationary phases, on the glass plates, were heated at 170°C for 60 min in an oven with precise temperature control.

Table I

The characteristics of the stationary phases investigated

Stationary phase notation	Merck catalogue number	Chemically bonded group
SiO ₂	5717	None
RP-2	5747	
RP-8	15388	
RP-18w	13124	
RP-18(1)	13724	
RP-18(2)	15389	

UV-Absorption Measurements

UV spectra in the wavelength range 200 to 350 nm were acquired with a Shimadzu (Columbia, MD USA) CS9301 PC scanning densitometer, using a rectangular cross-section light beam (dimensions 1 mm × 16 mm). The investigated chromatographic plates were used as the samples.

RESULTS AND DISCUSSION

In UV absorption spectroscopy the magnitude measured is usually the absorption of a sample. We assumed that changes in the intensity of the absorption at selected wavelengths caused by thermal modification was directly proportional to the relative density of coverage of the silica matrix with alkyl groups capable of aromatization. The ability of the adsorbent's alkyl groups to undergo thermally induced aromatization depends not only on there being a sufficient number of carbon atoms in the bonded chain (needed to build an unstrained aromatic ring) but also on steric effects and on different access of the individual alkyl groups to the catalytic agents (e.g.

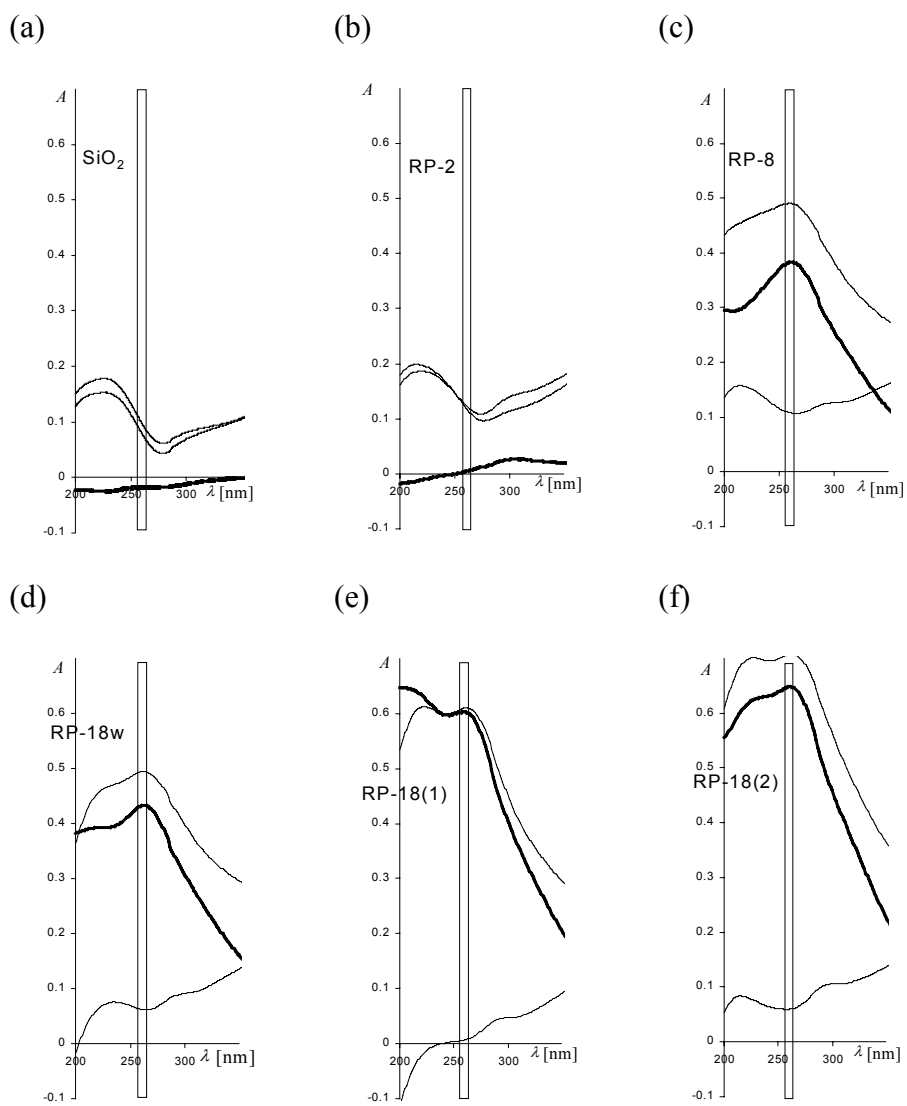


Fig. 1

UV spectra recorded for the unheated adsorbent (bottom line) and after heating the adsorbent for 60 min at 170°C (upper line), and the differential spectra (bold line). The range of maximum UV absorption in the differential spectra is indicated by the vertical bar. Samples: (a) SiO_2 , (b) RP-2, (c) RP-8, (d) RP-18w, (e) RP-18(1), and (f) RP-18(2)

the surface of the silica matrix). As is apparent from Fig. 1, all the stationary phases were characterized by recording their initial UV absorption spectra. Thermal treatment of the phases results in enhanced absorption. As a result of heating these stationary phases at 170°C for 60 min the increase in absorption observed depended on the relative density of coverage of the silica matrix with alkyl groups capable of aromatization. Because, in this study, the greatest increase of absorption was observed at 260 nm, this wavelength was taken as λ_{\max} , ensuring the greatest sensitivity of the analytical approach.

Let us assume that the intensity of Raman scattering in the range from ca 2750 to 1000 cm^{-1} depends on the density of coverage of the silica matrix with alkyl ligands and on the ability of the ligands to aromatize. In the wavenumber range indicated the medium-intensity Raman bands originating from the structural fragments of the aliphatic ligands are also present (Fig. 2 and Table II). These additional bands should not be integrated, because they are not related to aromatization, and should be removed from the Raman spectra before final calculation (Fig. 3)

In our study it was assumed the Raman scattering arising from the formation of aromatic compounds is entirely absent from the native silica gel samples and for this reason the Raman spectrum of the native silica was regarded as the baseline (Table III).

In our correlation study it was assumed that the intensity of the broad “aromatic” band is related to the density of coverage of the silica matrix with alkyl ligands capable of aromatization by the equation:

$$d = a \ln I + b \quad (1)$$

where d is the density of coverage of the silica matrix with alkyl ligands capable of aromatization, I is the Raman scattering intensity of the broad ‘aromatic’ band, and a and b are the equation constants. For systems which fulfil the Lambert–Beer law, the relationship between the density of coverage of the silica matrix with the ligands and the growth of UV absorption of stationary phases on heating at 170°C can be given by the equation:

$$d = pA + q \quad (2)$$

where A is the UV absorption and p and q are the equation constants. From these assumptions it follows that the magnitudes A and $\ln I$ should be linearly dependent and, therefore, our hypothesis can be verified by correlation by use of linear regression. It is apparent from Fig. 4 that there is a linear relationship between the growth of absorption at $\lambda = 260$ nm

induced by heating the stationary phases at 170°C for 60 min and the logarithm of the Raman scattering intensity in the wavenumber range from 2750 to 1000 cm^{-1} . The correlation coefficient, 0.997, is relatively high.

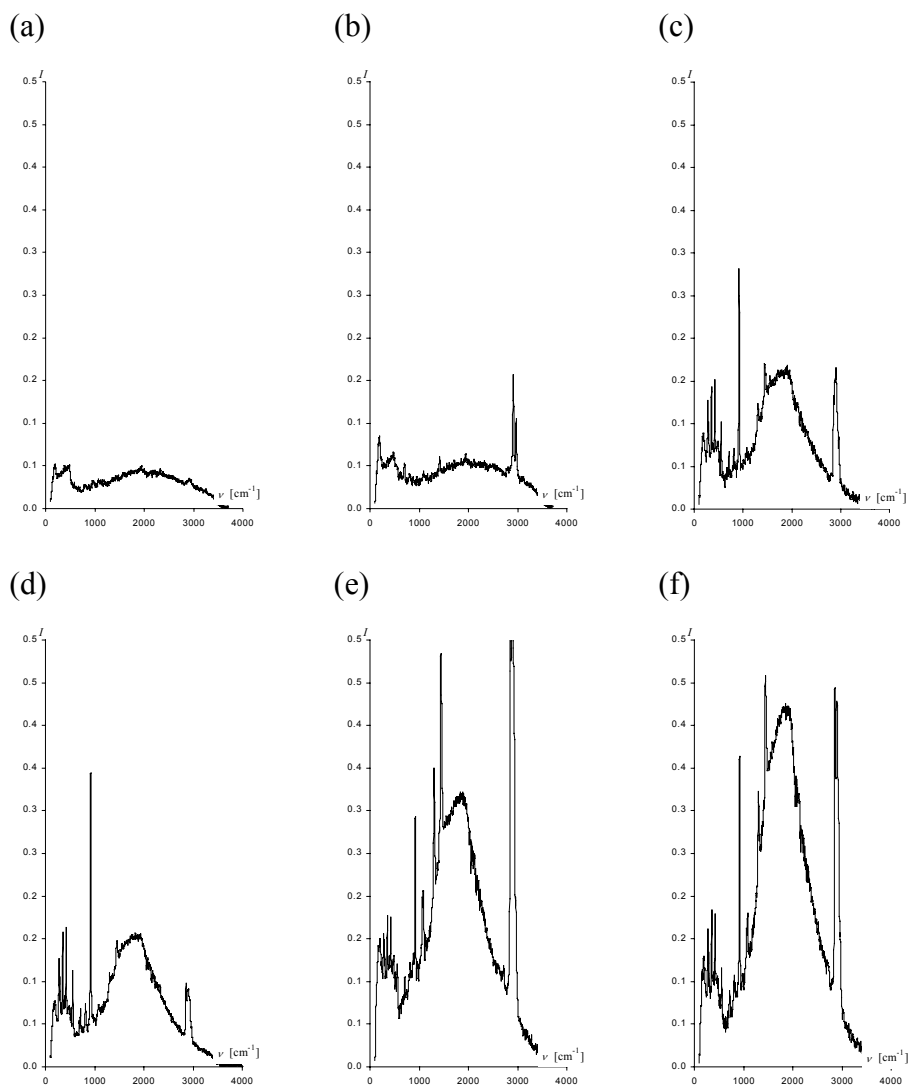


Fig. 2

Raman spectra recorded with use of the high-power neodymium laser source. Samples: (a) SiO_2 , (b) RP-2, (c) RP-8, (d) RP-18w, (e) RP-18(1), and (f) RP-18(2)

Table II

Characteristics of the medium-intensity Raman bands originating from the structural fragments of the aliphatic ligands according to data from Ref. [33]

Wavenumber (cm ⁻¹)	Assignment	Chemically bonded stationary phase				
		RP-2	RP-8	RP-18w	RP-18(1)	RP-18(2)
2965	$\nu_a(\text{CH}_3)$	++	++	–	++	++
2885	$\nu_a(\text{CH}_2)$	–	++	–	++	++
2855	$\nu_s(\text{CH}_2)$	–	++	++	++	++
1450	$\delta_a(\text{CH}_3)$	–	++	++	++	++
1420	$\delta(\text{CH}_2)_{\text{ortho}}$	+	+	–	+	+
1300	$\tau(\text{CH}_2)$	–	+	+	++	++
1080	$\nu(\text{C-C})_G$	–	–	–	+	+
1065	$\nu(\text{C-C})_T$	–	–	–	+	+

ν = stretch, τ = twist, δ = bend and/or scissor, $\delta(\text{CH}_2)_{\text{ortho}}$ = from orthorhombic crystals
s = symmetric, a = asymmetric
“–” = absent, “+” = least intense, “++” = most intense

CONCLUSIONS

This study of the correlation of data obtained from the two independent experimental techniques furnishes evidence of the good practical performance of the new method (based on the growth of the UV absorption of the stationary phase at $\lambda = 260$ nm which results from heating at 170°C for 60 min) for evaluation of the density of coverage of the silica matrix with alkyl ligands capable of aromatization. The results presented in this paper also justify our earlier hypothesis [31,34] that changes in the UV spectra are caused by thermal modification of the TLC adsorbent and are in evident proportion to the density of coverage of the silica matrix with alkyl ligands capable of aromatization. The growth in UV absorption at $\lambda = 260$ nm of stationary phases as a result of heating at 170°C for 60 min, can be linearly related to the data from Raman spectra reflecting the density of coverage of the silica matrix with alkyl ligands.

In the two approaches compared – Raman spectrometry and UV–visible spectrometry – the origin of the recorded signals is most probably analogous, i.e. thermal modification of the chemically bonded alkyl ligands. In Raman spectrometry this modification is because of heating of the adsorbent by irradiation with the high-energy laser beam. In the UV–visible spectrometry thermal modification of the ligands occurs at 170°C before

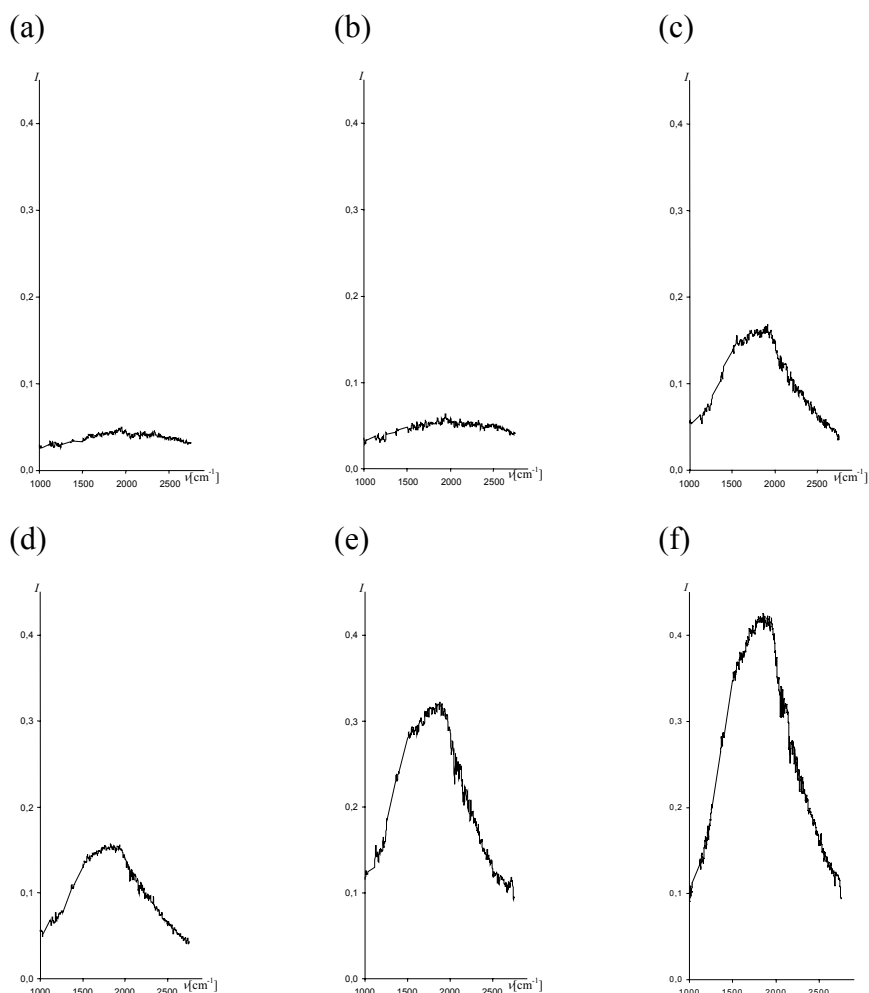


Fig. 3

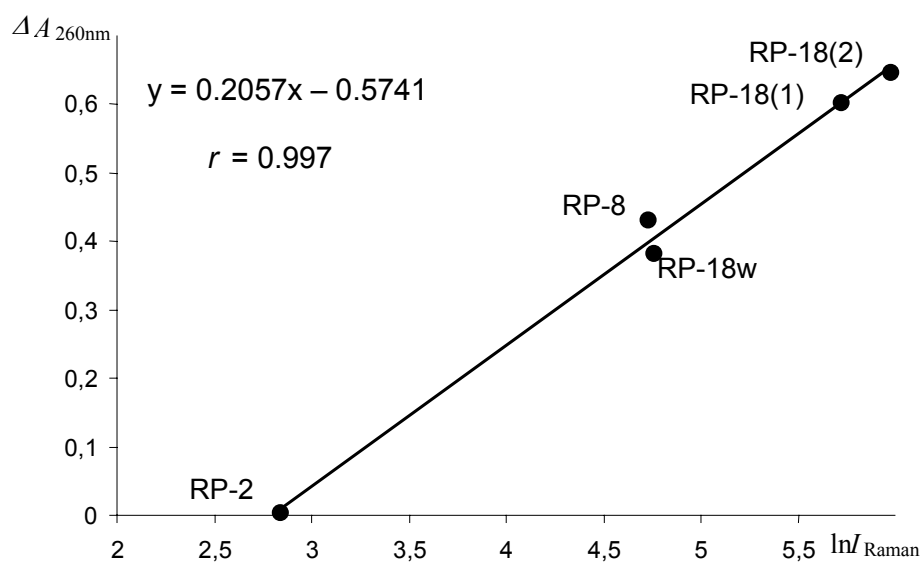
Raman spectra recorded with use of the high-power neodymium laser source. Samples (a) SiO₂, (b) RP-2, (c) RP-8, (d) RP-18w, (e) RP-18(1), and (f) RP-18(2) (all the spectra were preprocessed before integration)

acquisition of the spectra. The practical advantage of using UV absorption to monitor thermal modification of an alkyl-bonded stationary phase is the far wider access of chromatographers to UV–visible than to Raman equipment. Recording of UV–visible spectra is also possible with a typical TLC densitometer. Because UV–visible spectrometry is widely used for detection

Table III

Numerical values of selected UV and Raman spectral properties

Stationary phase	$\Delta A_{260\text{nm}}$	Raman $I_{2750-1000\text{ cm}^{-1}}$	Raman $\ln I_{2750-1000\text{ cm}^{-1}}$
SiO ₂	-0.019	baseline	—
RP-2	0.0043	17.08	2.838
RP-8	0.383	116.4	4.757
RP-18w	0.432	112.7	4.725
RP-18(1)	0.603	305.7	5.722
RP-18(2)	0.648	394.6	5.978

**Fig. 4**

Linear regression relationship between the growth of absorption at 260 nm and the logarithm of the Raman scattering intensity, integrated in the range from 2750 to 1000 cm^{-1}

in modern TLC, elaboration of a method powerful enough to quantify coverage of the silica matrix with aliphatic groups is a very promising and fairly readily available option.

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