

**CHANGES IN THE UV ABSORPTION OF SILICA  
CONTAINING ALIPHATIC MOIETIES  
AFTER THERMAL MODIFICATION,  
AND THE QUANTITATIVE RELATIONSHIP  
BETWEEN THE CHANGES AND THE ORGANIC  
GROUP CONTENT**

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**SUMMARY**

This paper reports an experiment performed to provide data for examination of the relationship between changes of stationary phase UV spectra as a result of thermal modification by heating at 165°C for 60 min and the content of alkyl groups. The results of a study on the quantitative relationship between coverage density of the silica matrix of a TLC-type stationary phase with an aliphatic moiety and its UV absorption after thermal modification are presented. Statistical analysis of the data obtained reveals a relatively high correlation between the increase in absorption and the paraffin oil content of impregnated RP-2 TLC plates. This report shows that evaluation of the aliphatic moiety content capable of aromatization in an impregnated stationary phase or on modified silica can be obtained solely by use of instrumentation typical of a TLC laboratory.

**INTRODUCTION**

The recently increasing popularity of the chromatographic techniques employed in analysis emphasizes the need to standardize the applied materials. Such a tendency results mainly from a desire to obtain reliable analytical results and to establish repeatable analytical procedures. So far, however, practical normalization of stationary phases, especially for reversed-phase liquid chromatography, seems difficult (or even impossible). Such difficulty follows mainly from the complex nature of these stationary phases (and particularly the so-called "surface phase"), which is mostly related to the synthetic methods used, which are characterized by a variety

of physicochemical conditions. Thus determination of the chromatographic properties of impregnated or chemically modified silica, based on the method of preparation, can lead to difficulties. For chemically modified silica the quality of the chemically bonded groups and their quantity per unit of the adsorbent surface seems to be significant, as also is the quantity and type of polar group (e.g. free silanols) [1,2]. A search for novel methods of stationary phase assessment seems fully justified, to expand sources of information on structural features influencing the mechanism of retention.

A new method of relative evaluation of the coverage density of the silica matrix with octyl, octadecyl, 3-cyanopropyl, and diol ligands has been introduced [3–6]; it is based on the *Raman* spectroscopy (and more specifically on an intense and broad *Raman* band of obscure origin). Determining the origin of this finely structured, intense and broad band in the frequency range from ca. 2285 to 1130  $\text{cm}^{-1}$  proved a real challenge. Systematic study of the behavior of the chemically bonded stationary phases of interest (RP-8, RP-18, CN, diol and RP-2 with the paraffin oil impregnation) at elevated temperatures, employing differential scanning calorimetry (DSC), UV and IR spectroscopy, and modified acquisition of *Raman* spectra has been described [7]. To confirm the formation of aromatic products in chemically bonded stationary phases heated at 165°C, extracts obtained from washing these pretreated adsorbents were further analyzed by HPLC–DAD [8]. The aim of this study was to prove the existence, and then determine the quantitative dependence of the content of aliphatic groups in the TLC-type stationary phase and the UV spectral changes observed as a consequence of thermal modification. To achieve this goal, several tasks were performed:

- impregnation of the ready-made stationary phase with paraffin oil and a precise monitoring of the extent of impregnation by weighing the plates;
- determination of the number of alkyl groups on the stationary phases by measurement of the mass increase after impregnation;
- recording UV–visible spectra of the samples of stationary phases with different amounts of aliphatic groups before and after thermal modification;
- computing difference spectra for pairs of samples before and after thermal modification; and
- studying correlations between difference spectra and aliphatic group content.

A novelty of this study is that it provides direct proof of the quantitative dependence of changes of the UV absorption caused by thermal modification and the content of alkyl groups capable of aromatizing on the silica matrix of the TLC-type stationary phase. This is additional evidence of the properties of stationary phases after impregnation, or of the modified silica, as described elsewhere [3–8].

## EXPERIMENTAL

### The Stationary Phases Used, and Thermal Modification

The study was performed on ready made RP-2 chromatographic glass plates manufactured by E. Merck (Darmstadt, Germany; TLC plates precoated with silanized silica gel 60 F<sub>254</sub>, Merck #5747) and similar plates impregnated with 2.5, 5, 10, 25, and 50% solutions of paraffin oil in *n*-hexane by dipping them in the impregnating solution and then drying them for 24 h at ambient temperature. The *n*-heptane used as solvent was obtained from Reachim (USSR).

For thermal modification of the stationary phases the glass plates were heated at 165°C for 60 min in a temperature-controlled oven.

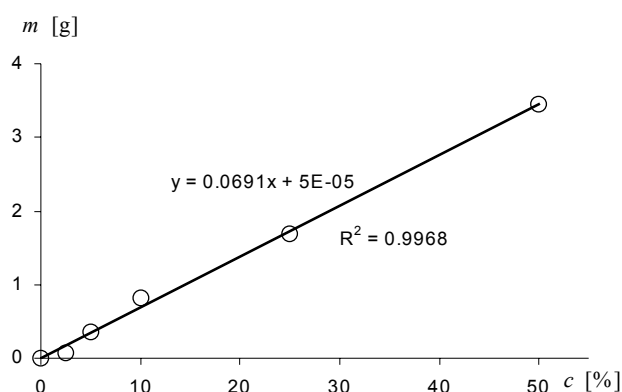
### UV–Visible Spectrometry

UV–visible spectra in the wavelength range 200 to 600 nm were recorded, using the transmittance and the reflectance modes, by means of a Hitachi model U2001 spectrophotometer fitted with a Labsphere RSA-HI-20 integration system. Transmittance mode, with a quartz cell of path length 5 cm was used to check for contamination of *n*-heptane and paraffin oil by aromatic compounds. The reflectance mode using Spectralon White as reference was employed for investigation of the stationary phases. For the latter investigation the stationary phase samples were normally prepared as four independent specimens (for each concentration of the impregnating solution) and they were examined both as fragments of the glass plate and the scraped off powder, placed in a quartz cell with the path length of 1 cm.

### Data Collection and Processing

To detect possible aromatic contamination of solvents the absorption spectra of *n*-heptane and paraffin oil were recorded (experimental conditions are given above). In the paraffin oil only there was a relatively low-intensity band with  $A_{\max} = 0.1$  in the wavelength range from 310 to 345 nm.

The amount of paraffin oil impregnating the stationary phase was determined gravimetrically. The result obtained was given as a weight of paraffin oil per 0.04 m<sup>2</sup> of the original chromatographic plate. Dependence of the paraffin oil content in the chromatographic bed on its concentration in the impregnating solution was established for each stationary phase sample and analyzed statistically, considering the individual sample values and the average values (Fig. 1).

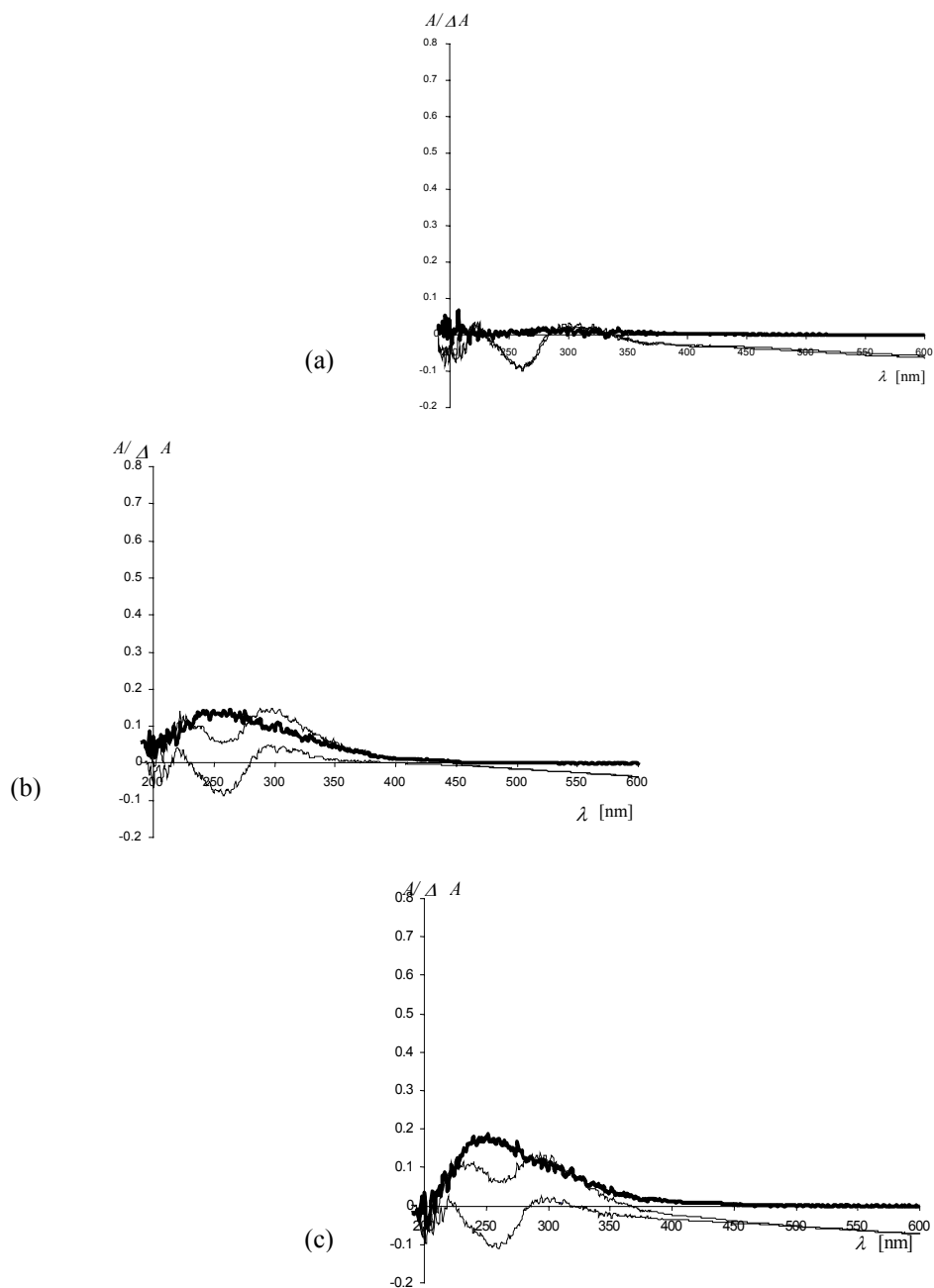


**Fig. 1**

Dependence of the total paraffin oil content of the chromatographic bed on a 20 cm × 20 cm chromatographic plate on the concentration of the impregnating solution

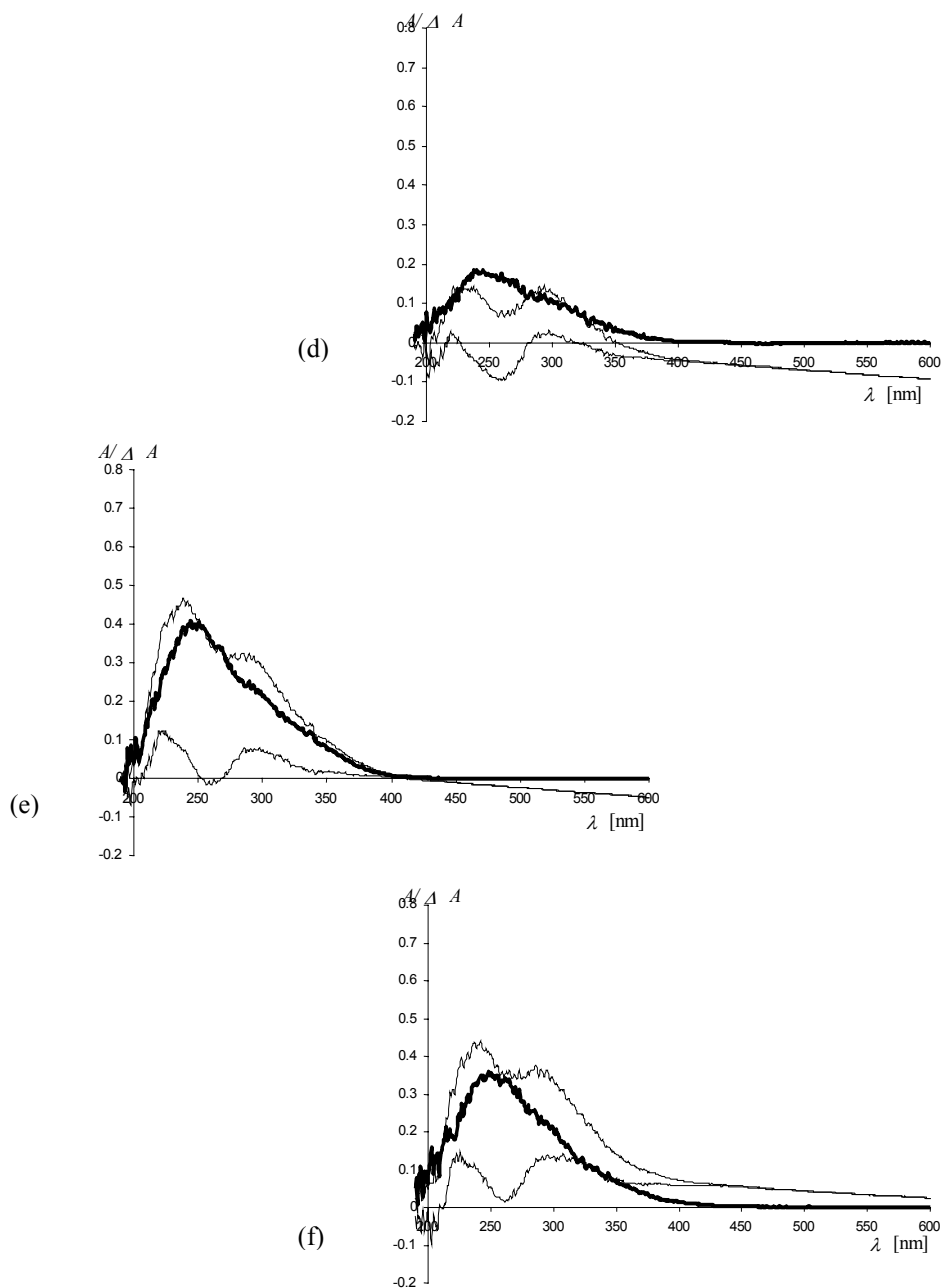
The UV–visible spectra of the heated samples (obtained as described above) had to be numerically pretreated to achieve normalization. The starting point for this pretreatment was the assumption that between the unheated and heated samples there should be no absorption difference at 600 nm. In practice, a randomly shifting baseline of the measured spectra was observed, which had to be overcome. Thus the pretreatment procedure consisted in superposition of the UV–visible spectra for a pair of unheated and heated stationary phase samples solely at 600 nm. To evaluate the effect of heating, difference spectra (i.e. differences between absorption of unheated and heated samples) were calculated from the pretreated original spectra (Figs 2 and 3).

Quantitative relationships were established between the changes in the spectra caused by heating and the different paraffin oil content, employing either the integrated absorption intensities of the difference spectra in the wavelength range 210 to 330 nm ( $\Delta A_{210-330}$ ) or using the absorption intensities taken from the difference spectra at 250 nm ( $\Delta A_{250}$ ).



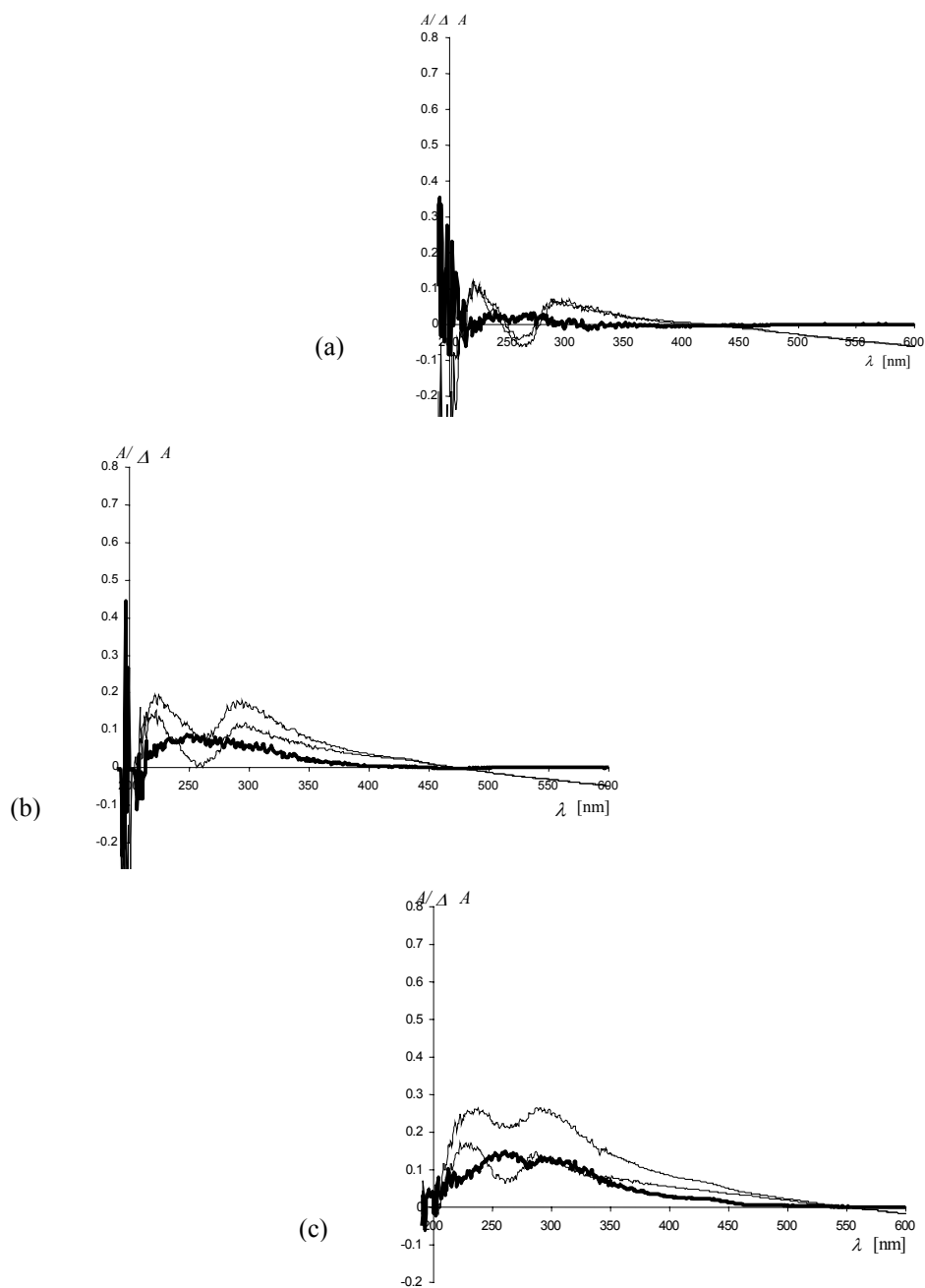
**Fig. 2**

UV spectra recorded from RP-2 plates (a) without impregnation and impregnated with (b) 2.5%, and (c) 5% paraffin oil solution; unheated (bottom line), heated for 60 min at 165°C (upper line), and the difference spectrum (thickened line)



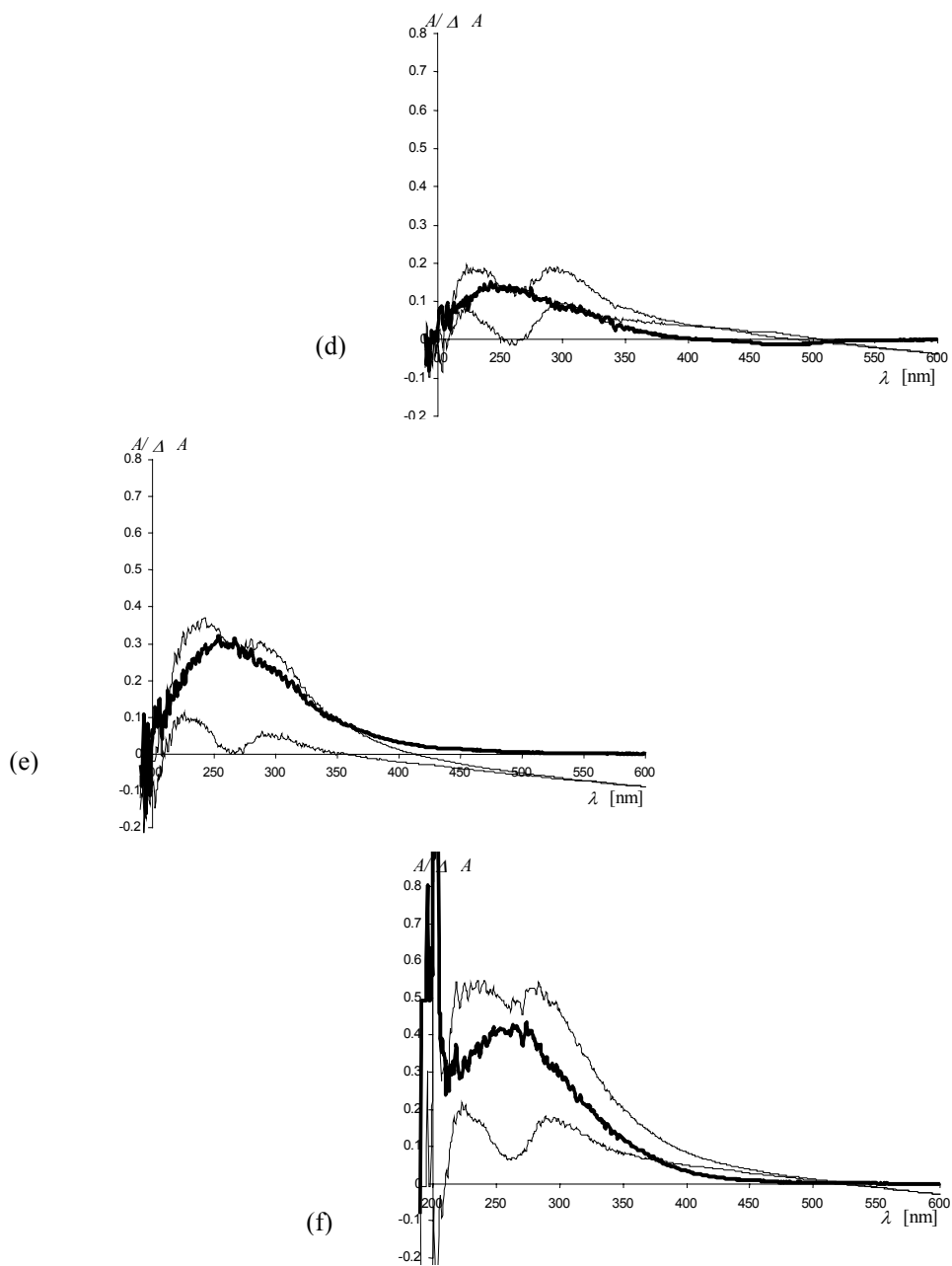
**Fig. 2 (continued)**

UV spectra recorded from RP-2 plates impregnated with (d) 10%, (e) 25%, and (f) 50% paraffin oil solution; unheated (bottom line), heated for 60 min at 165°C (upper line), and the difference spectrum (thickened line)



**Fig. 3**

UV spectra recorded for pulverized RP-2 stationary phase samples (a) without impregnation and impregnated with (b) 2.5%, and (c) 5% paraffin oil solution; unheated (bottom line), heated for 60 min. at 165°C (upper line), and the difference spectrum (thickened line)



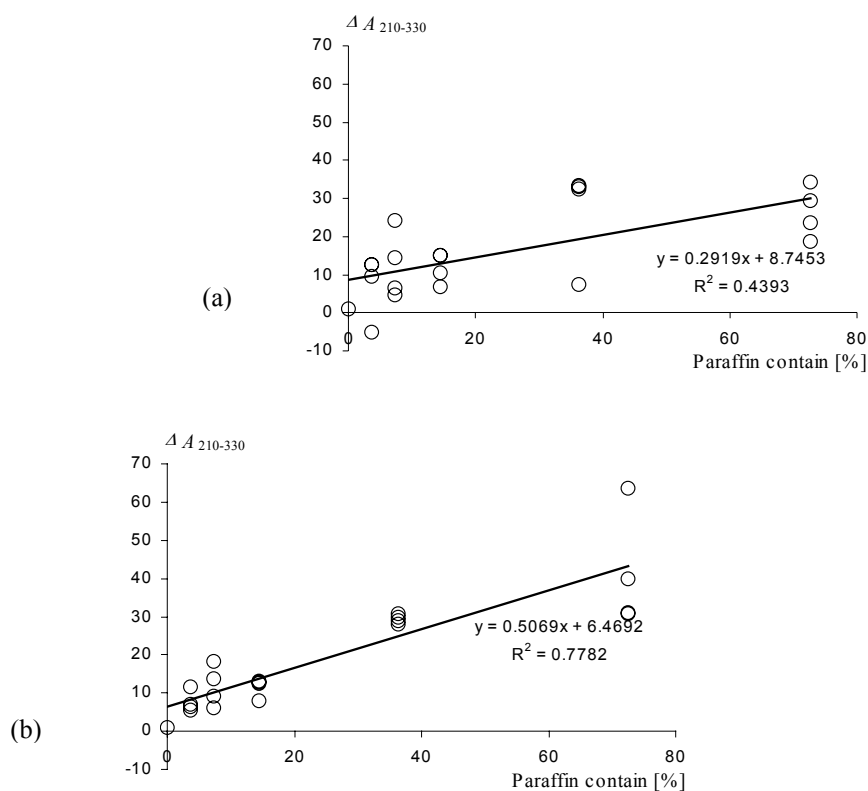
**Fig. 3 (continued)**

UV spectra recorded for pulverized RP-2 stationary phase samples impregnated with (d) 10%, (e) 25%, and (f) 50% paraffin oil solution; unheated (bottom line), heated for 60 min. at 165°C (upper line), and the difference spectrum (thickened line)



## RESULTS AND DISCUSSION

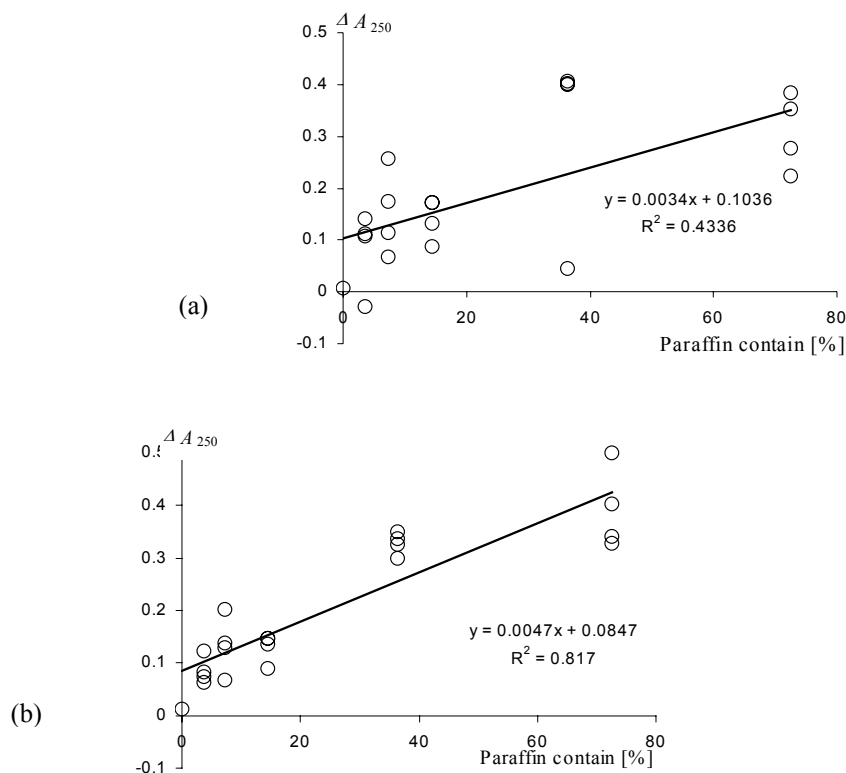
On the basis of data obtained by weighing samples of the paraffin oil used for impregnation (as described above), numerical values of the squared correlation coefficient  $R^2$  were computed for the linear dependence of liquid paraffin content of the chromatographic bed on its concentration in the impregnating solution. The numerical values of  $R^2$  for data from all the samples and for data averaged for each sample treated with the same concentration of impregnating solution were 0.64 and 0.98, respectively. This discrepancy of the results can either be caused by an uneven distribution of the paraffin oil in the stationary phase or uneven thickness of the adsorbent on the glass plate.



**Fig. 4**

Dependence on paraffin oil content of the increase in absorption integrated in the wavelength range 210 to 330 nm for (a) chromatographic plates and (b) adsorbent powder

The results presented in this paper support the hypothesis [3–8] that changes in the UV spectra are caused by thermal modification of the stationary phases and that the extent of the changes is in proportion to the paraffin oil content of the RP-2 phase. This study on the quantitative dependence of the alkyl moiety content in stationary phase and UV–visible absorption as a result of thermal treatment apparently shows an increase in the intensity of absorption in the wavelength range from 210 to 330 nm (and particularly at 250 nm) with increasing paraffin oil content in chromatographic bed. Dependencies shown in Figs 4b and 5b (established from spectra of the pulverized stationary phase in a quartz cell) have much higher  $R^2$  values than analogous dependencies obtained by use of data from experiments with the samples deposited on the glass bed (Figs 4a and 5a).



**Fig. 5**

Dependence on paraffin oil content of the increase in absorption at 250 nm, for (a) chromatographic plates and (b) adsorbent powder

The respective  $R^2$  values are most probably an effect of the matrix, because the non-homogeneity of the chromatographic bed surface and the microporosity of sample cause specific and nonspecific light scattering. It is apparent that crumbling the stationary phase reduces this effect. With the plate samples, an additional cause of the measurement errors may be the presence of the glass under the stationary phase layer; this probably absorbs some of the light of interest for this study.

Although Spectralon White is employed as a reference, the baselines of the recorded spectra are unstable and usually have to be corrected. Integration works best for wavelengths close to 300 nm. At lower wavelengths the performance decreases [9].

Correction of the baseline position (which depends on lowering the entire spectral profile by an amount depending on the different absorption intensities at 600 nm) partially removes the baseline instability and hence eliminates its impact on the results from statistical analysis. The highest value of  $R^2$  ( $>0.8$ ) was obtained for the linear dependence between the difference between absorbance at 250 nm and the liquid paraffin content of the samples in the quartz cell (Fig. 5b).

The quantitative dependence of changes in UV absorption (caused by thermal modification) on the number of alkyl groups on the silica matrix capable of aromatization makes a considerable contribution to description of the properties of impregnated stationary phases or modified silica. The results obtained in this study show the possibility of routine quantification of the content of alkyl groups capable of aromatization on the silica matrix. For further evidence of the usefulness of this method it should be compared with another approach to the same problem, integration of the high-energy laser *Raman* band in the wavelength range 2285 to 1130  $\text{cm}^{-1}$  [3–6].

A practical advantage of the method described in this paper is the more ready availability of UV–visible equipment than *Raman* spectrometers. Recording of UV–visible spectra is also possible with a typical TLC densitometer. Because UV–visible spectrometry is widely applied in the TLC densitometry, its use for quantifying coverage of the silica matrix with aliphatic groups is a very promising and a relatively readily available option.

## CONCLUSIONS

- The increase in absorption of stationary phases as a result of heating for 60 min at 165°C is a linear function of the aliphatic group content of the silica matrix.
- Although Spectralon White was used to fix the reference baseline of the recorded spectrum, additional correction was required.
- Relatively low squared correlation coefficients ( $R^2$ ) were obtained by use of data obtained from spectra recorded from the unprocessed chromatographic plates; substantially better results were obtained by pulverizing the stationary phases and recording the spectra by use of a quartz cell.

Further study of the quantitative relationship between changes in the UV spectra caused by thermal modification of the paraffin oil-impregnated silica matrix or of chemically bonded with aliphatic groups, requires a comparison with the results from another reliable method.

## REFERENCES

- [1] W. Prus, Y. Vander Heyden, P. Vankeerberghen, P. Kuś, D.L. Massart, and T. Kowalska, *Chemom. Intell. Lab. Syst.*, **47**, 253 (1999)
- [2] W. Prus, *The Jubilee Volume, The XXXth Anniversary of The Technical University of Łódź Branch in Bielsko-Biała, Bielsko-Biała, 1999*, pp. 346–349
- [3] G. Kowalik, K. Rogosz, and T. Kowalska, *J. Am. Off. Anal. Chem. Assoc. Int. (JAOAC Int.)*, **82**, 297 (1999)
- [4] G. Kowalik, K. Rogosz, K. Bojarska, and T. Kowalska, *J. Planar Chromatogr.*, **12**, 429 (1999)
- [5] G. Kowalik, B. Klama, R. Okopska, and T. Kowalska, *J. Planar Chromatogr.*, **13**, 25 (2000)
- [6] G. Kowalik and T. Kowalska, *J. Planar Chromatogr.*, **13**, 348 (2000)
- [7] W. Prus, J. Fabia, C. Dobosz, B. Szlosarczyk, A. Strządała, and T. Kowalska, *J. Planar Chromatogr.*, **14**, 378 (2001)
- [8] W. Prus, M. Sajewicz, and T. Kowalska, *J. Planar Chromatogr.*, **15**, 324 (2002)
- [9] Technical documentation of Labsphere's RSA-HI-20 Reflectance Spectroscopy Accessory for the Hitachi U-2000 spectrophotometer