Photoacoustic Investigations of Secondary Chromatographic Effects on TLC Plates

Irena Vovk[1], Mladen Franko[2]*, Jürgen Gibkes[3], Mirko Prošek[1] and Dane Bicanic[3]

[1] *National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia,*

[2] *School of Environmental Sciences, P.O.B. 301, SI-5001 Nova Gorica, Slovenia, and*
Jožef Stefan Institute, Jamova 39, SI-1111 Ljubljana, Slovenia

[3] *Wageningen Agricultural University, Dept. of Agricultural Engineering and Physics, Bomenweg 4,*
6703 HD Wageningen, The Netherlands

Photoacoustic spectroscopy (PAS) was used to study the vertical concentration distribution of compounds on TLC plates. The results suggest that under the given experimental conditions compounds exhibit irregular concentration profiles in vertical direction and tend to concentrate in the uppermost 61 μm thick layer. This irregularity was accounted for by photoacoustic probing of thicker sorbent layers what lead to an improved linearity of calibration curves compared to those obtained by reflectance densitometry.

Keywords Photoacoustic spectroscopy, TLC, densitometry, thermal diffusivity, depth profiling

The TLC plates are usually investigated by reflectance scanning densitometry in the UV spectral region. However, such measurements are restricted only to the surface of the TLC plate, and do not take into account the vertical sub-surface concentration distribution governed by the secondary chromatography within the sorbent[1-4]. This phenomenon, occurring during the drying process of TLC plates, may lead to the erroneous interpretation of TLC chromatograms obtained by reflectance densitometry. It is therefore important to investigate effects associated with secondary chromatography in order to reveal their origin, and to estimate how they affect the results of TLC measurements.

Photoacoustic spectroscopy (PAS), that relies on the detection of pressure waves (sound) generated by the absorption of radiation in a periodically irradiated sample, has been previously used for qualitative and quantitative spectroscopic analysis of TLC plates[5]. Likewise, the PAS was demonstrated a good candidate method for depth profiling of solid samples[7-10]. This unique feature of PAS is due to the fact that the magnitude of the induced photothermal effect, in addition to the concentration of a particular compound, also depends on the thermal diffusivity of the sample. Therefore, the plot showing the dependence of the photoacoustic (PA) signal on the modulation frequency provides the information about the depth related profile of the sample. However, if one is to determine the thickness of the probed layer, thermal diffusivity of the tested sample must be accurately known. The unavailability of thermal diffusivity values for TLC plates requires their determination by means of an experiment. This was already demonstrated in our previous work[11-13] which suggests that the PAS is the most suitable approach among the photothermal techniques for characterization of TLC plates. It is for this very reason, that the PAS was used here to obtain vertical concentration profiles of compounds on TLC plates.

Experimental

TLC

TLC was performed on 5×5 cm glass-backed TLC plates (Merck, Darmstadt, Germany) coated with a 0.25 mm layer of Kieselgel 60 F_{254}. Test dye mixture III (Camag, Muttenz, Switzerland), which contains oracet 2R, indophenol, ariabel red, sudan blue II, oracet red G and dimethyl yellow, was diluted with toluene (Kemika, Zagreb, Croatia) to obtain 20%,

60%, and 80% solutions. Diluted and non diluted solutions of test dye mixture III were applied to the sorbent layer in spots using 0.5 µl glass capillaries and the Nanomat III applicator (Camag, Muttenz, Switzerland). The plates were developed in an un-saturated glass flat-bottom chamber (Camag) using toluene as a mobile phase. After separation, the plates were dried in a stream of warm air for about 5 min.

Densitometric evaluation of the developed plates was performed using the Camag TLC scanner II (set to reflectance mode) equipped with a built-in 12 bit ADC, and controlled by an external PC via an RS232 interface. The QTLC-pack (KIBK-IFC, 1990) software was used. The bandwidth of the monochro-mator was 30 nm, the slit width 0.6 mm and the slit length 4 mm. All measurements were performed at $\lambda=520$ nm.

PAS measurements

All PA measurements were performed using the experimental set up described in detail elsewhere[14]. In most cases measurements were performed by scanning the modulation frequency of an argon-ion laser (tuned to the 514.5 nm emission line) from 1 Hz to 10 kHz. A plate of Sigradur G glass, a surface absorber with well known thermal properties, served as a reference material for normalization of signals and corresponding phase lags. A Quartz plate (8 mm thick) was used to correct the PA signal for unwanted effect of stray light.

The PA signals were analyzed using the theory for a two-layer sample[15] consisting of the sorbent (surface layer) and the glass as the supporting material. The thermal diffusivity values α of the spots on TLC plates were obtained by curve fitting of normalized phase lags of PA signals at different modulation frequencies.

Different thickness of the sample, corresponding to the thermal diffusion length μ, given as $\mu = \sqrt{(\alpha /\pi f)}$, were probed by varying the frequency of laser beam modulation.

Results and Discussion

To study the effects of inhomogeneities in TLC plates and the effects of varying concentration of the test dye mixture, sets of TLC chromatograms were prepared. Several spots containing identical concentration of the test dye mixture were applied to different TLC plates. Furthermore, spots of different

concentrations were applied to one and the same TLC plate.

Thermal diffusivity values obtained by the curve fitting of data for normalized phase-lag of PA signal to the two layer model ranged from $(25\pm2)\times10^{-6}m^2s^{-1}$ to $(35\pm2)\times10^{-6}m^2s^{-1}$ for 20% to 100% test dye mixture, respectively. These values were later used to calculate the thickness of the substrate layers (thermal diffusion lengths) probed at different modulation frequencies, and to extract the PA signals originating from different depths of TLC plates.

The PA signals originating from different layers of a TLC plate were calculated by subtracting the values obtained at higher modulation frequencies from those obtained at lower modulation frequencies. From these two modulation frequencies and the previously obtained thermal diffusivities of each spot, the depth and the thickness of each layer were calculated. Depending on the available range of modulation frequencies used in the PA measurements the thickness of the probed layers varied from 22 to 58 μm. The results corrected for differences in layer thickness are shown in Figs. 1 to 4.

It is evident that in all cases the compounds remained concentrated in the upper layer 58 or 61 μm thick. A difference between the PA signals from different TLC plates (Fig. 1) which exceeds the typical 6% experimental error for the described measurement procedure was observed. Despite identical concentration of compounds in the investigated spots, the measurements on plate 3 (Fig. 1) produced consistently higher PA signal (compared to two other plates) throughout the entire sorbent.

At higher modulation frequencies the depth reso-lution for the upper layer increases and the differences in vertical concentration distribution of
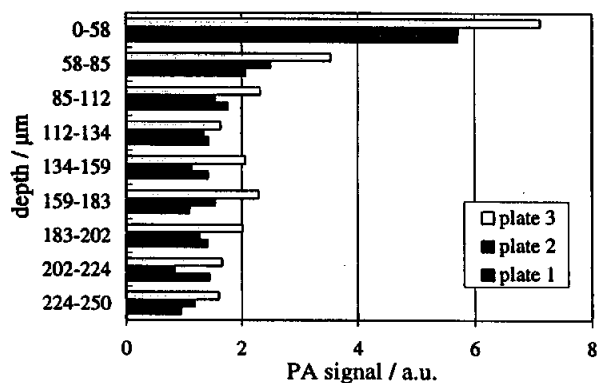

Fig. 1 Depth distribution of the compound in yellow spots of equal concentration applied to different TLC plates
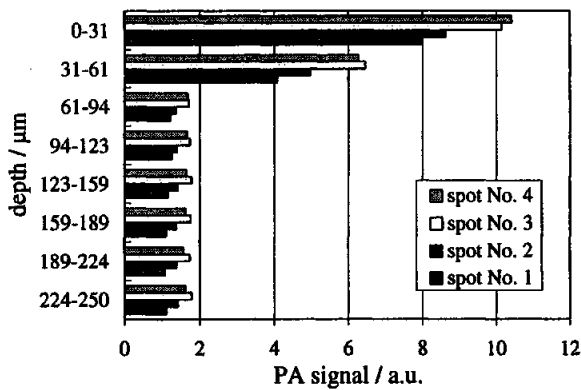
Fig. 2 Depth distribution of the compound in yellow spots of equal concentrations.
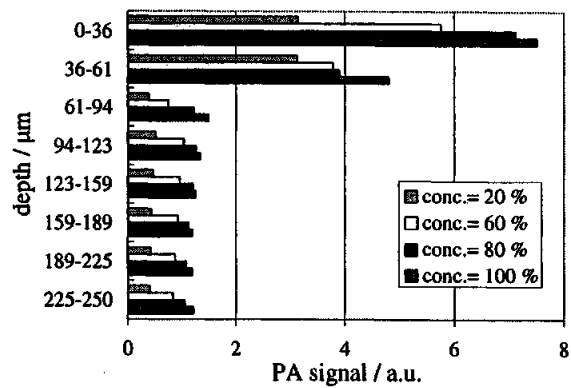

Fig. 4 Depth distribution of the compound in yellow spots of different concentration.

different compounds from the same plate could be observed (Figs. 2 and 3). For the same reasons a non linear calibration curve is obtained when the probed sorbent layer is too thin (Figs. 5 and 6).

While the measurements on the yellow spot show higher concentration of the compound in the top 31μm layer, the concentration of the compound in the violet spot is higher in the region between 39 and 61 μm. Furthermore, differences in PA signals from the same depths are observed for different spots indicating that inhomogeneity within one and the same TLC plate could be the source of erroneous quantitation of TLC chromatograms.

The effects discussed above were also observed when different concentrations of test dye mixture were applied to the same TLC plate (during the preparation of calibration curves). As shown in Fig. 4 the vertical distribution of the compound in the spots of 20 % test dye mixture in the top two layers is comparable while at higher concentrations of test dye

mixture the compound concentrates within a layer 0 - 36 μm thick. In addition, relative ratios of signals from the 0 - 36 μm and 36 - 61 μm layers for compounds applied at various concentrations are different.

Probing only the surface of the TLC plate, such as in the case of the reflectance densitometry (Fig. 5) can therefore result in the nonlinearity of calibration curves. For example, when the selected modulation frequency corresponded to thermal diffusion lengths equal or shorter than 36 μm, the calibration curve was not linear because of different vertical distributions of compounds in spots corresponding to different concentrations of test dye mixture (top curves on Fig. 6). Once the thickness of probed layer is sufficient to account for all considerable irregularities in vertical concentration distribution (61 μm), the correlation between the measured PA signal and the concentration of the test dye mixture becomes linear as expected (lower curves on Fig. 6)
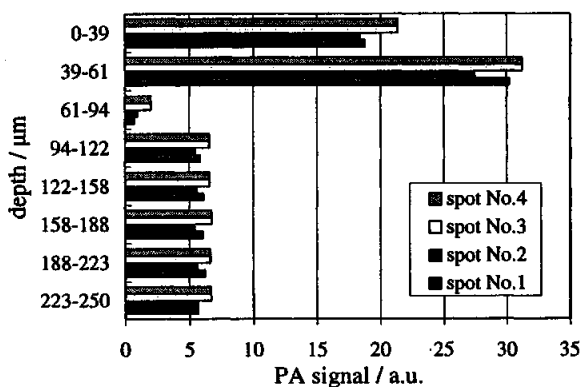

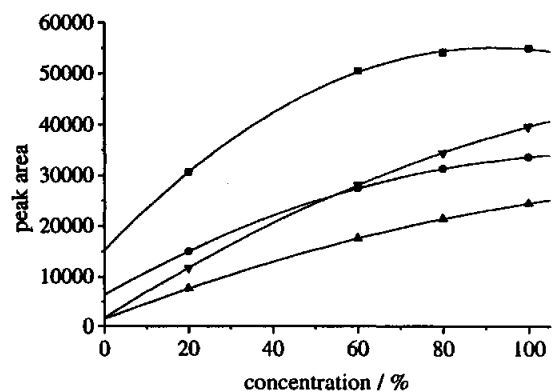Fig. 3 Depth distribution of the compound in violet spots of equal concentration.

Fig.5 Calibration curves for the Camag test dye mixture III (■ red spots, ● blue spots, ▲ violet spots, ▼ yellow spots) obtained by reflectance densitometry.
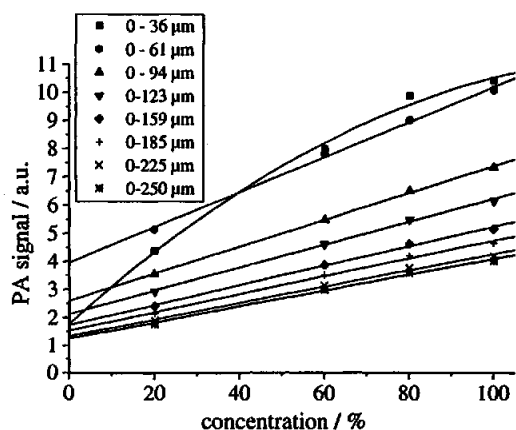
Fig. 6 Calibration curves for the yellow spots obtained by the PAS when probing a different thickness of the sorbent.

Conclusions

The results of our PA investigations on TLC plates indicated that under the specified chromatographic conditions, the largest concentration of the tested compound is found in the surface layer not exceeding 61 μm of the substrate. The differences in vertical distribution of concentration in different spots can be attributed to the inhomogeneity of the sorbent on a given chromatographic plate and to the differences in various TLC plates. Such irregular vertical distribution of concentrations which can lead to the nonlinearity of calibration curves can be compensated by photoacoustic probing of thicker sorbent layers. By virtue of its capacity to identify the inhomogeneities in the vertical distribution of compounds within the sorbent, and by actually detecting compounds of interest inside the entire layer of the sorbent, the PAS is not hindered by the effects of secondary chromatography. In this context the PAS is more reliable technique for quantification of TLC plates when compared to the reflectance densitometry, which measures the concentrations only at the surface of a TLC plate.

Due to the smaller radius of laser beam used for excitation (compared to sizes of the spots on TLC plates) the reported results do not exclude the possibility of varying radial distribution of concentrations within the sorbent, which on its turn might also lead to corresponding irregularities of PA signals. Application of PAS to study the radial concentration distribution on TLC plates is foreseen in the near future.

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