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Photothermal characterization of thin-layer chromatography plates[1]

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Abstract

The potential of photothermal beam deflection spectrometry (PTDS), photoacoustic spectroscopy (PAS) and photothermal radiometry (PTR) for the characterization of thin-layer chromatography (TLC) plates with respect to the surface and in-depth distribution of different compounds inside the sorbent was investigated. Photothermal measurements of TLC chromatograms of the Camag III test dye mixture demonstrate that accurate and comparable values for thermal diffusivity of spots in chromatograms and of the sorbent can be obtained by PTDS and PAS. PAS has been demonstrated to be the most reliable and least time-consuming among the three investigated techniques. Differences observed between the PAS signal and the phase-frequency scans obtained from the spots of the same compound on different TLC plates could be due to the irreproducibility of chromatogram development or the inhomogeneousness in different TLC plates. © 1997 Elsevier Science B.V.

Keywords: Photothermal characterization; Photothermal beam deflection spectrometry; Photoacoustic spectroscopy; Photothermal radiometry; Thermal diffusivity; Thin layer chromatography

1. Introduction

Most of the densitometric measurements of thin-layer chromatography (TLC) plates are performed in the UV spectral region by applying reflectance scanning densitometry. However, the results of densitometric reflectance measurements are strongly dependent upon the depth distribution of the analysed material [1]. This has also been proven by using special multi-layer models [2–4]. Nonuniform concentration distribution of the compound inside the sorbent is the main reason for the low reproducibility of TLC measurements. Such nonuniform concentration distribution is mainly due to the secondary chromatography inside the sorbent, which occurs

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during drying of the plate. Even by carefully controlling the chromatographic conditions, these effects can only be partly eliminated. It is therefore our objective to investigate the applicability of alternative densitometric techniques such as photothermal beam deflection spectrometry (PTDS), photoacoustic spectroscopy (PAS) and photothermal radiometry (PTR) for characterization of TLC plates with particular emphasis on the surface and in-depth distribution of compounds inside the sorbent.

The techniques used in this work belong to the group of photothermal techniques that are based on the detection of heat induced phenomena in an irradiated sample. The amount and the rate of dissipation of generated heat can be detected by measuring the deflection angle of the probe laser beam (PTDS), the intensity of the sound produced by heating of the gas contacting the sample (PAS), or by measuring the intensity of the IR radiation emitted by the heated area of the sample (PTR). In addition to the concentration of a particular compound, the magnitude of the induced photothermal effect also depends on the sample's thermal properties such as for example thermal diffusivity α and thermal effusivity e. Furthermore, the signal's dependence on the modulation frequency contains information about the depth related profile of the sample.

PTDS and PAS in particular have been frequently used for qualitative and quantitative spectroscopic analysis of TLC plates [5–11]. It is also very well known that, owing to their physical characteristics, photothermal techniques can be used for depth profiling of solid samples [12–15]. Recently, the depth profiling ability of PAS, coupled with the imaging capabilities of the stationary two-dimensional Hadamard encoding mask, enabled three-dimensional imaging of TLC plates [16]. The reported images corresponded to the bulk, surface and subsurface sample layers. However, for depth profiling with higher resolution, thermal diffusivity of the sample must be accurately known if one is to determine the thickness of the probed layer. As in many other cases, thermal diffusivity values of various spots and the sorbent on TLC plates are not known and must be determined experimentally. In this work PTR, PTDS and PAS were used to determine the thermal diffusivity needed to investigate the in-depth distribution of analyte compounds on a TLC plate.

2. Theory

When radiation interacts with a solid sample, part of the radiation energy may be converted into heat. For a non-fluorescing sample the conversion efficiency is close to unity and the corresponding amplitude of the surface temperature T_0^* of a heated semi-infinite solid is related to the incident radiation flux I_0 via the simple relationship:

$$I_0 = T_0^* \sqrt{2\pi f}\ \sqrt{k\rho c} \tag{1}$$

where $e = \sqrt{k\rho c}$ is the thermal effusivity of the sample and k, ρ and c are thermal conductivity, density and heat capacity of the sample, respectively.

Periodically modulated irradiation of the sample results therefore in an oscillating temperature of the sample. Such temperature oscillations are termed thermal waves. These can be represented by thermal wavelength $\lambda = \sqrt{(4\pi\alpha/f)}$ and by the propagation velocity (defined as $v = \lambda f$) of temperature maxima or minima, where $\alpha = k/\rho c$ and f represent thermal diffusivity of the sample and the frequency of modulation, respectively. Characteristic for thermal waves is a strong amplitude damping and a phase lag $\Delta\varphi = x\sqrt{(\pi f/\alpha)} + \pi/4$ between periodically varying heating flux and the resulting thermal response. The phase lag that increases with the propagation distance x can also be expressed in terms of thermal diffusion length μ defined as $\mu = \sqrt{(\alpha/\pi f)}$. Since both μ and $\Delta\varphi$ vary with modulation frequency of the heating process, proper selection of f and the knowledge of α enables one to selectively probe different thicknesses of the sample.

Because of heat conduction, contact gas surrounding the irradiated sample is also heated. Consequently, thermophysical properties of the gas change resulting in various effects, which in turn can be exploited in different ways to measure the amount of absorbed energy. For example, the thermal wave response of the solid, induces a pressure oscillation $\delta p(t)$ in the contacting gas confined within a constant volume, which can be detected by a microphone. Such detection schemes are used in PAS, and the magnitude of pressure oscillation is given by the following expression [17,18]:

$$\delta p(t) = \frac{\gamma - 1}{l_g} \int_0^t F(\tau)\mathrm{d}\tau \tag{2}$$

where $F(\tau)$ is the periodic surface heat flow from the sample to the gas. In Eq. (2) γ and l_g represent the adiabatic coefficient of the gas and the length of the cell, respectively. In general $F(t)$ and the one-dimensional temperature distribution $T(x,t)$ are related through [17,18]:

$$F(t) = -k\nabla T(x,t) \tag{3}$$

However, in layered samples, effects of thermal reflection at different layers must be taken into account. For a two-layer model [19] consisting of the surface layer, i.e., pure sorbent or sorbent containing a chromatographic spot (subscript s), and supporting material in a TLC plate, i.e., glass (subscript b), the magnitude S_n of the normalized photoacoustic signal is given by:

$$S_n\sqrt{f} = \frac{\sqrt{(k\rho c)_s}}{\eta_s}\left[\frac{1-R_{sb}e^{-2\sqrt{\pi f}\Lambda_s}\left[2\cos(2\sqrt{\pi f}\Lambda_s)-R_{sb}e^{-2\sqrt{\pi f}\Lambda_s}\right]}{1+R_{sb}e^{-2\sqrt{\pi f}\Lambda_s}\left[2\cos(2\sqrt{\pi f}\Lambda_s)+R_{sb}e^{-2\sqrt{\pi f}\Lambda_s}\right]}\right]^{1/2} \times\frac{\eta_r\beta_r}{\sqrt{2\pi}\rho_r c_r}\left[1+\sqrt{\frac{\alpha_r}{\pi}}\beta_r f^{-1/2}+\frac{\alpha_r}{2\pi}\beta_r^2 f^{-1}\right]^{-1/2} \tag{4}$$

In Eq. (4) S_n is defined as a ratio of amplitudes of photoacoustic signals S_r and S obtained from the reference specimen (subscript r) and the sample under the test, i.e., $S_n = S_r/S$, and η_s is photothermal conversion efficiency of the surface layer. Furthermore, $\Lambda_s = l_s/\sqrt{\alpha_s}$ is the square root of thermal diffusion time for thickness l_s of the surface layer and the thermal reflection coefficient R_{sb} for the two-layer model is defined by: $R_{sb} = \{\sqrt{(k\rho c)_s}/\sqrt{(k\rho c)_b} - 1\}/\{\sqrt{(k\rho c)_s}/\sqrt{(k\rho c)_b} + 1\}$. The normalized phase lag $(\varphi_r - \varphi_s)$ of the photoacoustic signal described by Eq. (4) is then given by:

$$\cot(\varphi_r-\varphi_s)\sqrt{f} = \frac{0.5\sqrt{f}}{\sin(2\sqrt{\pi f}\Lambda_s)}\left[\frac{e^{2\sqrt{\pi f}\Lambda_s}}{(-R_{sb})} + R_{sb}e^{-2\sqrt{\pi f}\Lambda_s}\right] \tag{5}$$

Hence, thermal diffusivity as well as thermal effusivity values of the substrate on a TLC plate can be obtained by curve fitting of experimental data for the phase lag to Eq. (5).

In addition to the acoustic pressure (Eq. (2)), the heat generated at the surface of a solid material produces a refractive index gradient in the contacting gas or other fluid surrounding the sample. This can be probed by measuring the deflection of a laser beam skimming the surface of the heated sample. The vertical (transverse) component of deflection angle Θ_z experienced by the probe beam along a distance L can be approximately given by [20]:

$$\Theta_z = \left(\frac{L}{n}\right)\left(\frac{dn}{dT}\right)\left(\frac{T_0^*}{\mu_g}\right) \tag{6}$$

where n and (dn/dT) are the refractive index of the gas (usually air) and its temperature coefficient, subscript z refers to the displacement perpendicular to the plane of the sample and μ_g is thermal diffusion length in the contacting gas.

The measurements of transverse deflection angle and phase lag performed at different modulation frequencies and probe beam offsets enable one to obtain thermal diffusivity of a solid sample [21,22]. In this work the so called "phase method", a variant of the PTDS technique, was used for determination of α. This method relies on the computation [22] of a slope m' from experimental plot displaying the phase as a function of the vertical offset (i.e., a distance between the surface of the heated sample and the probe beam) at a given modulation frequency. This slope is related to the thermal diffusivity by:

$$m' = \sqrt{\frac{\pi f}{\alpha}} \tag{7}$$

In addition to the effects described above, the periodically varying temperature $\delta T(x,t)$ of a solid sample leads to the corresponding radiant emittance changes δW [23] determined by the Stefan–Boltzmann law:

$$\delta W = 4\varepsilon\sigma T^3\delta T(x,t) \tag{8}$$

where σ and ε are the Stefan–Boltzmann constant and the emissivity of the sample (an ideal grey body at temperature T). This radiant emittance is superimposed upon substantially larger but continuous radiant emittance $W = \varepsilon\sigma T^4$.

Assuming a sinusoidal excitation and normal incidence of radiation, the temperature oscillation $\delta T(x,t)$ in an opaque, semi-infinite slab of a material is [20]:

$$\delta T(x,t) = \frac{1}{2} \frac{\eta I_0 e^{-\frac{x}{\mu}}}{\sqrt{k\rho c}\sqrt{2\pi f}} \cos(2\pi ft - \Delta\varphi) \quad (9)$$

Using a suitable broad band infrared detector the PTR signals can be recorded as a function of modulation frequency. The analysis of both amplitude and phase of PTR signals enables one to derive meaningful information about optical (conversion efficiency η and optical absorption coefficient β) and thermal properties (thermal effusivity e and thermal diffusivity α) of the sample.

3. Experimental

3.1. TLC

TLC was performed on 5×5 cm glass-backed HPTLC plates (Merck, Darmstadt, Germany) coated with a 0.25 mm layer of Kieselgel 60 F_{254} or Kieselgel 60. Test dye mixture III (Camag, Muttenz, Switzerland) was applied to the layer in spots using 0.5 μl glass-capillaries and the Nanomat III applicator (Camag). The plates were developed once in an unsaturated glass flat bottom chamber (Camag) using toluene (Kemika, Zagreb, Croatia) as a mobile phase. After separation the plates were dried in a stream of warm air for about 5 min.

Evaluation of the developed TLC plates was performed densitometrically using the Camag TLC scanner II equipped with a built-in 12 bit ADC, and controlled by an external personal computer (PC) via an RS232 interface. The QTLC-pack (KIBK-IFC, 1990) software was used. The scanner was set to reflectance mode. The monochromator bandwidth was 30 nm, the slit width 0.6 mm and the slit length 4 mm.

3.2. Photothermal measurements

Photoacoustic, photothermal beam deflection and IR radiometric measurements were all performed using experimental setups described in detail previously [19,23,24]. All measurements were performed at the 514.5 nm emission line of an argon ion laser for the range of modulation frequencies varying from 1 Hz to 10 kHz. Sigradur G glass, a surface absorber, was used as a reference material for normalization of signals and corresponding phase lags. An 8-mm thick quartz plate was used to compensate for unwanted effect of stray light in photoacoustic measurements.

4. Results and discussion

The results presented in this section were obtained from photothermal measurements performed on the sorbent and on violet and yellow spots from TLC chromatograms. The distribution of spots on TLC plates is evident from the densitogram shown in Fig. 1.

Typical PAS and PTR frequency scans corresponding to the two spots and the sorbent from the densitogram (Fig. 1) are shown in Fig. 2. As expected from the absorption properties of investigated materials, the highest normalized signal S_n, (i.e., signal from Sigradur G relative to the signal obtained from the sample) was observed for the sorbent followed by the signal for the yellow spot. The lowest S_n values were obtained for the violet spot on the TLC plate (A). Differences between the two spots and the TLC plate sorbent are also evident from the phase plots (B) which are not affected by the differences in absorbance (Eq. (5)). As the phase lag is not sensitive to η, the observed differences must be arising from different thermal diffusivities of the sorbent and the investigated spots. A similar trend is observed in the results of PTR measurements (C).

Both PA and PTR measurements provide consistent results indicating that the sample under the

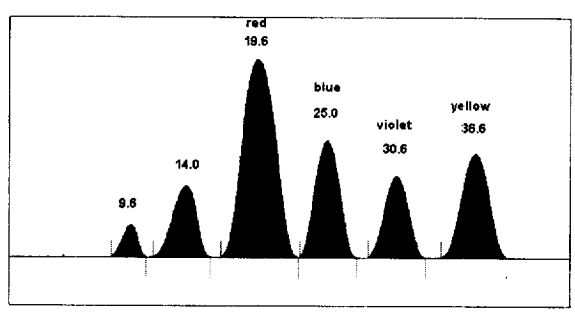

Fig. 1. Densitogram obtained for Camag test dye mixture III at $\lambda = 520$ nm. Numbers indicate the migration distance in mm.

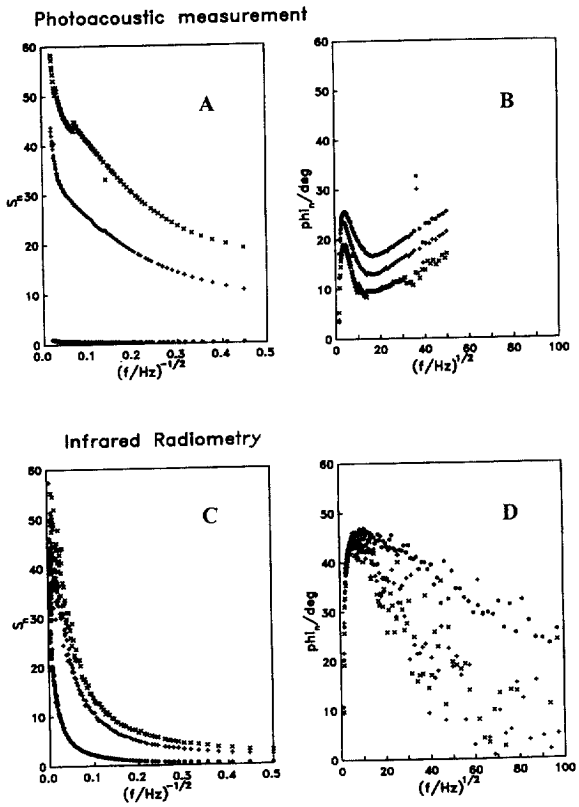

Fig. 2. Normalized frequency dependent amplitude (S_n) and the phase (φ_n) photothermal signals obtained by the PAS (A, B) and PTR (C, D) on the sorbent (×), yellow (+) and violet (*) spots from one TLC plate (Kieselgel 60 F_{254}).

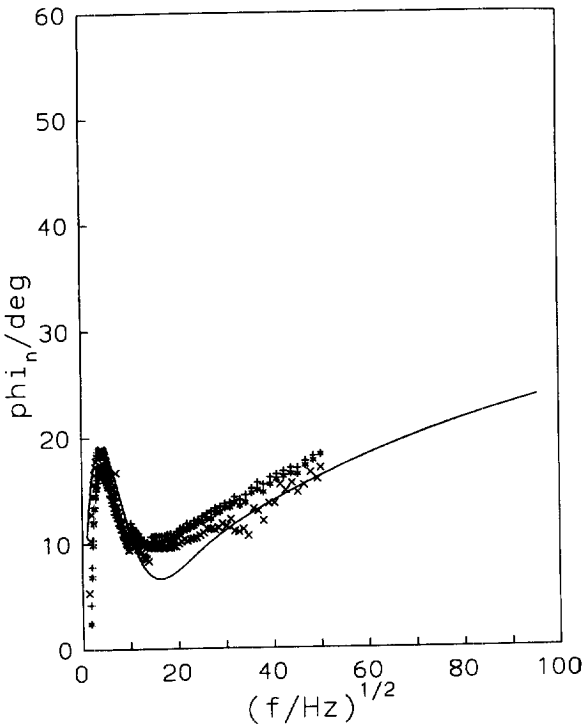

Fig. 3. Normalized frequency dependent phase signals obtained by the PAS for the sorbent on three TLC plates of the same type (Kieselgel 60 F_{254}). Solid line is the best fit to Eq. (5) for one set of values (×).

test is not completely optically opaque to the 514 nm excitation wavelength of an argon ion laser. However, differences in phase lag plots obtained by three PTR measurements are difficult to be observed, because of the much higher noise compared to the PAS measurements. To some extent this is due to intrinsically lower sensitivity of the PTR technique. Furthermore, the lack of validated PTR theoretical models, which take into account the effect of varying absorption of emitted IR radiation within the sample, at present precludes the fitting of data obtained from actual PTR measurements.

To obtain thermal diffusivities, results of PAS experiments were fitted to Eq. (5) described previously. Fig. 3 shows three phase plots obtained in PAS measurements of a white sorbent region from three TLC plates. Agreement among the three sets of values demonstrates good reproducibility of the measurements and reveals actually no difference in thermal diffusivities of investigated TLC plates. Similar results were also obtained for violet and yellow spots on the TLC chromatogram (Figs. 4 and 5) selected for testing the applicability of the PAS for thermal diffusivity measurements and depth profiling of TLC plates. However, unlike the results obtained for the white sorbent (Fig. 3) and violet spots (Fig. 4), the differences in values for yellow spots (Fig. 5) suggest some variation in homogeneity of substrates on the four TLC plates. Larger dispersion of yellow spots additionally contributes to the observed differences.

The solid lines on Figs. 3–5 represent the best fit to a selected set of data. Thermal diffusivity values obtained from curve fitting procedures are summarized in Table 1 together with the thermal effusivity values, and show good agreement with values ob-

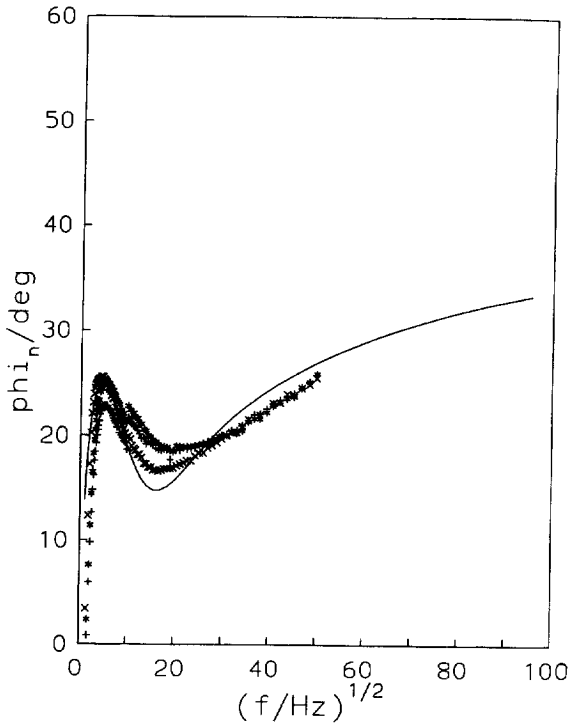

Fig. 4. Normalized frequency dependent phase signals obtained by the PAS for violet spots from three TLC plates of the same type (Kieselgel 60 F_{254}). Solid line is the best fit to Eq. (5) for one set of values (×).

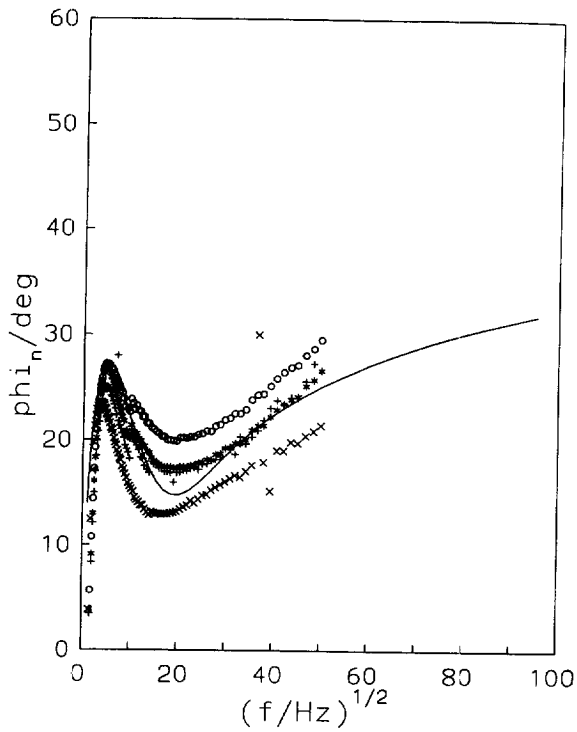

Fig. 5. Normalized frequency dependent phase signals obtained by the PAS for yellow spots from four TLC plates of the same type (Kieselgel 60 F_{254}). Solid line is the best fit to Eq. (5) for one set of values (*).

tained by the phase variant of the PTDS method. The experimental error in thermal diffusivity for samples listed in Table 1 is typically 6% and 20% for PAS and PTDS measurements, respectively.

Although capable of providing absolute values of thermal diffusivity for both, thermally thick and thermally thin samples in a non-contact manner, PTDS is time consuming mainly due to the need for signal acquisition at several probe beam displacements. This and a considerably larger standard deviation were the main reason why PTDS was not further used in our studies.

The differences in thermal properties of various measured spots are evident in Table 1. It can also be noticed that in case of the yellow spot the values of thermal diffusivity and effusivity are higher compared to those obtained from the violet spot. The lowest values were always obtained from the white sorbent.

Using thermal diffusivity values obtained from PAS measurements we have calculated that thermal diffusion length of 250 μm, which equals the thickness of the sorbent layer, corresponds to frequencies of 90 Hz for the white sorbent and violet spot, and 125 Hz for the yellow spot. As seen in Figs. 3–5, at these frequencies, the decreasing part of the phase lag plot begins to bend before reaching a local minimum. This observation is an additional argument for the validity of PAS results obtained with investigated TLC plates.

In addition to studies of different TLC plates, the effects of different concentrations of the test dye mixture applied to one and the same TLC plate were investigated. As shown in Fig. 6, no significant differences were observed for four yellow spots of equal concentration on the same TLC plate. However, a 20% change in concentration can clearly be observed when comparing the fits for two yellow

Table 1
Thermal diffusivities and effusivities of different regions of a TLC plate as measured by PTDS and PAS

Probed spot	Technique		
	PTDS	PAS	PAS
	$\alpha/10^{-6}$ ($m^2 s^{-1}$)	$\alpha/10^{-6}$ ($m^2 s^{-1}$)	$\sqrt{k\rho c}/10^3$ ($W s^{-1/2} m^2 K^{-1}$)
White sorbent	15±4	18±1	4.0±0.2
Yellow spot	22±4	25±2	6.0±0.4
Violet spot	–	18±1	5.2±0.3

spots of different concentration from one TLC plate (Fig. 7). This was also reflected in about 20% difference in thermal diffusivities calculated from the two fits. These experiments indicate that the concentration changes associated with secondary chromatographic effects could be detected and the resulting errors in quantification of TLC chromatograms eliminated by applying PAS for depth profiling of TLC plates and previous measurement of their thermal diffusivity.

5. Conclusions

The results of photothermal studies of TLC plates

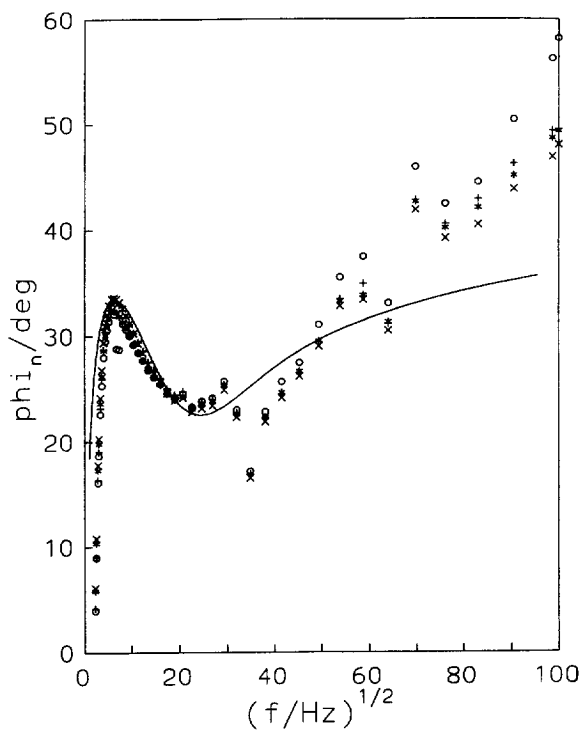

Fig. 6. Normalized frequency dependent phase signals obtained by the PAS for four yellow spots (100% Camag III) from one TLC plate (Kieselgel 60). Solid line is the best fit to all data points.

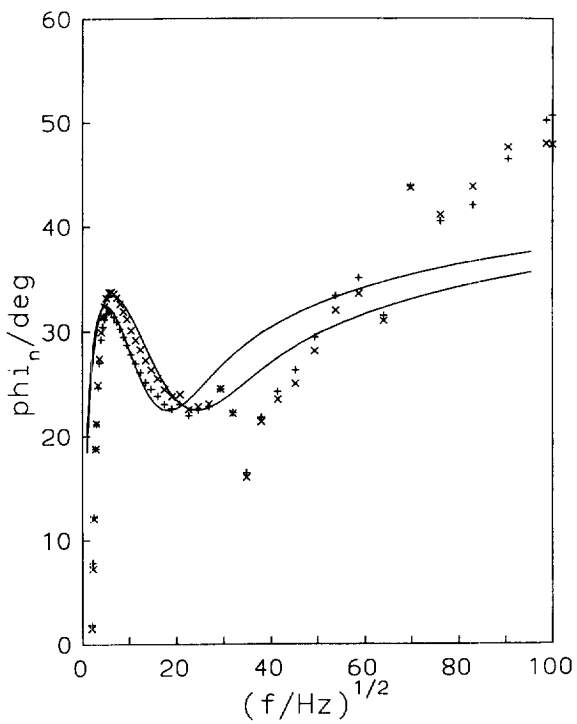

Fig. 7. Normalized frequency dependent phase signals obtained by the PAS for two yellow spots of different concentrations (×: 100% Camag III; +: 80% Camag III in toluene) from one TLC plate (Kieselgel 60). Solid lines are the best fits to each set of values.

using PAS, PTDS and PTR methods suggest that PAS is the most convenient technique in terms of sensitivity, accuracy and the time needed for completing the analysis. PAS enables reliable determination of thermal diffusivity values for the sorbent and the chromatographic spots, which depend primarily on the concentration of the sample applied to the TLC plate.

In our future work thermal diffusivity values measured by the PAS will be used to select the range of appropriate modulation frequencies and the associated thermal diffusion lengths for mapping of depth dependent concentration profiles across the chromatographic spots on TLC plates. Such measurements could lead to an experimental confirmation of the theoretical predictions about the secondary chromatographic effects in TLC [3,4] and provide the basis for better interpretation of densitometric results obtained during quantification of TLC chromatograms.

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