

Quantitative estimation of substances spotted on filter paper by photoacoustic spectroscopy⁺

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MS received 8 January 1987

Abstract. Quantitative estimation of coloured as well as colourless substances spotted on filter paper has been carried out by means of photoacoustic spectroscopy. The technique should be specially useful in the estimation of biological substances.

Keywords. Photoacoustic spectroscopy; quantitative estimation of spots on filter papers.

1. Introduction

Advantages of photoacoustic spectroscopy (PAS) in the study of materials, normally inaccessible to conventional optical spectroscopic techniques, have been described by various workers in the last few years (Rosencwaig 1980; Ganguly and Rao 1981; Tam 1986). Opaque and light scattering materials such as powders have been studied by Rosencwaig (1975). Adams *et al* (1980) have studied the quantum efficiency of solid fluorescent materials. Phase transition studies have been carried out by Florian *et al* (1978) and Somasundaram *et al* (1986a). Recently, Jagannathan *et al* (1982) have shown that PAS can be used for the study of surface acidities of solid oxide catalysts. Busse and Orgaback (1980) have used PA effect for depth profiling studies. Even biologically important materials have been studied by PAS. For example, Cahen *et al* (1978) have studied photosynthesis of chloroplast membranes; Balasubramanian *et al* (1984) have studied malaria parasites; Somasundaram *et al* (1986b) have studied the pigments in the thermophilic fungi.

The technique has been used for qualitative estimation of substances adsorbed on thin layer chromatograms by Rosencwaig (1975). Achwal *et al* (1984), however, have carried out quantitative estimation of 5-methylcytosine present in the DNA of *Drosophila melanogaster* by making use of a sensitive immunochemical assay and PA spectroscopy. These authors have used nitrocellulose paper for spotting the DNA and analysed the spectra for estimating the amount present. We considered it most worthwhile to investigate whether PAS was suitable for the quantitative estimation of substances spotted on ordinary filter papers, since a successful demonstration of such an application would open up a new dimension in analytical methods, especially in the case of biological substances. Estimation of substances spotted on filter paper would also serve to demonstrate one of the quintessential advantages of PA spectroscopy.

⁺ Contribution No. 378 from the Solid State and Structural Chemistry Unit.

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2. Experimental

A single beam PA spectrometer described earlier elsewhere by Ganguly and Rao (1981) was used for this study. The light source was a 450 W high pressure xenon lamp. The light beam was modulated by a mechanical chopper and focussed into the cell by an adjustable mirror so that various areas of the sample could be investigated. For all the spectra obtained the conditions were as follows: scanning speed 100 nm/min; spectral resolution 20 nm; and chopping frequency 37Hz. The sample holder was modified in such a way as to accommodate filter papers without

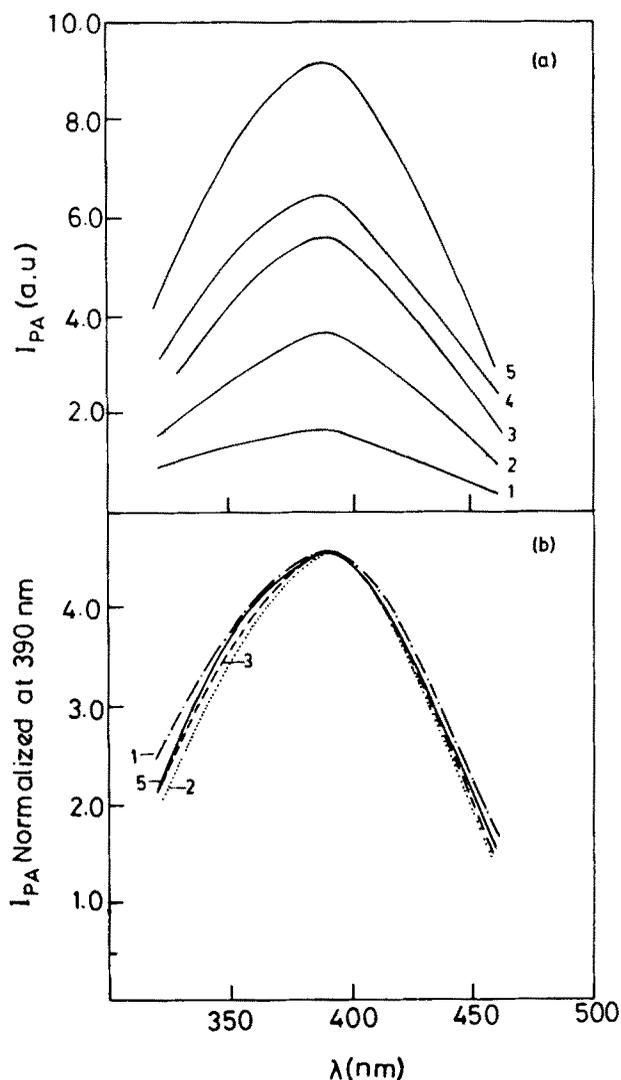


Figure 1. a) Normalized PA spectra of different amounts of *p*-nitroaniline spotted on Whatman-41 filter paper. 1: 1×10^{-3} M; 2: 2.5×10^{-3} M; 3: 5.0×10^{-3} M; 4: 7.5×10^{-3} M; 5: 1×10^{-2} M. b) Same spectra after equalizing intensities at 390 nm.

appreciable loss in signal. As usual normalization of recorded spectra were carried out using carbon black power spectrum.

Filter papers used were Whatman-40, 42 and 41. The filter paper was spotted using a micro syringe using either aqueous or alcoholic solutions of the substances. The diameter of the spots did not exceed 3 mm (illumination covered the spots fully). Different concentrations of solutions were used for spotting different amounts, keeping the quantity of liquid spotted the same for one series of experiments (either 2 or 5 μl). Different volumes of the solutions of the same substance, containing the same weight of substance were also spotted to see whether there was any volume effect. For *p*-nitroaniline and other UV absorbing species, spotting was done either under UV light or on cut-out pieces of filter paper. Blank filter paper pieces were used to record background contribution and they were subtracted from the signal at every wavelength before reporting. The compounds studied were *p*-nitroaniline, crystal violet, neutral red, cytosine and L-tyrosine.

3. Results

In figure 1a we show the carbon black normalized PA spectra of *p*-nitroaniline at various concentrations spotted on Whatman-41 filter paper after subtracting background signals due to the filter paper. After equalizing the intensities of various spectra at 390 nm (corresponding to various concentrations) the same spectra are replotted in figure 1b. One sees that all the spectra are superimposable. Similar results are obtained for the substances studied and the normalized PA spectra of all of them are shown in figure 2.

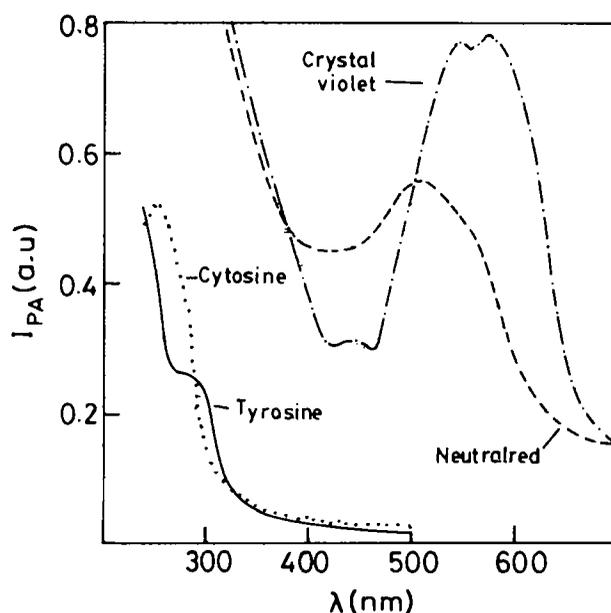


Figure 2. Normalized PA spectra of some compounds spotted on Whatman-41 filter paper.

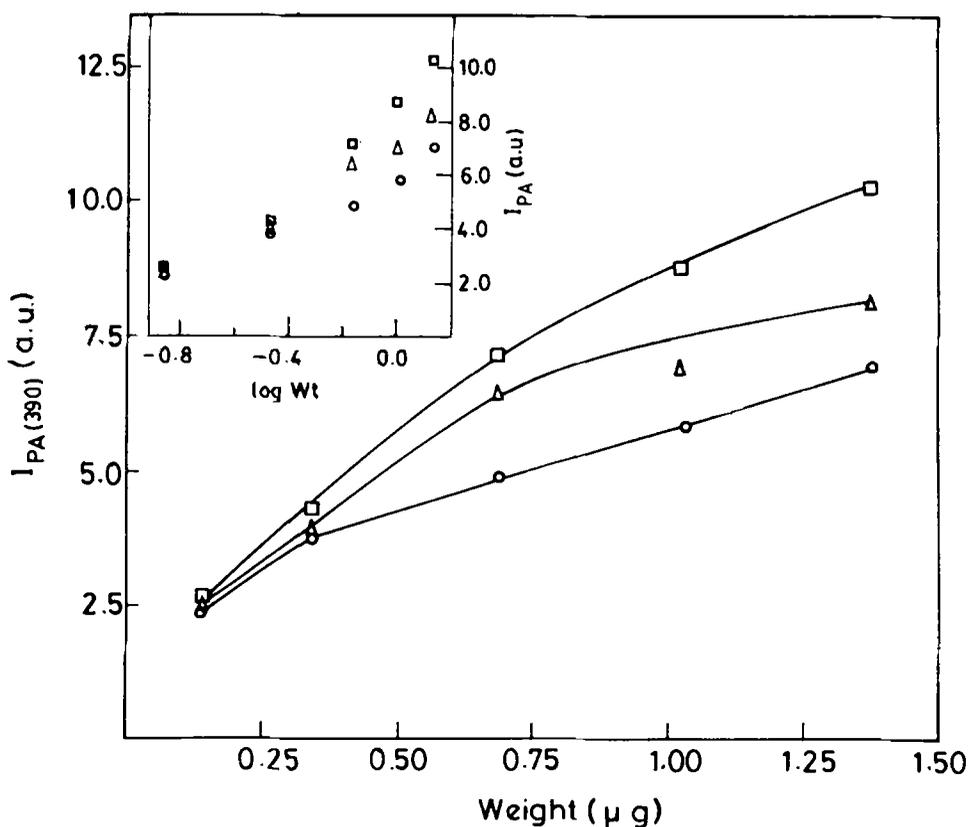


Figure 3. I_{PA} vs weight plot of *p*-nitroaniline spotted on three different filter papers. Circles, Whatman-41; triangles, Whatman-40; squares, Whatman-42. Inset shows I_{PA} vs log weight plots of the same spots.

In figure 3 we show the variation of PA intensity (I_{PA}) of *p*-nitroaniline at 390 nm for various amounts spotted on three different types of filter paper. We note that the intensities are not linearly related to the concentrations. Secondly, the intensity is greater when the filter paper is of finer grain size. For coarse grain filter paper, I_{PA} vs weight (wt.) of the substance, the plot is nearly linear in the high concentration regime. In figure 4 we show the variation of I_{PA} vs. wt. for other substances spotted on Whatman-41 filter paper. One sees a linear relationship between the intensity and concentration.

4. Discussion

The present study shows that there is a definite proportionality of I_{PA} to the wt. of the substance spotted on filter paper. To quantify this relationship, PA spectra has to be checked for saturation effects, as otherwise, increase in the amount may not bring about an increase in I_{PA} . It is seen from figure 1b that all the spectra are superimposable on one another indicating that even at the highest concentration studied there is no sign of saturation; furthermore, background subtraction

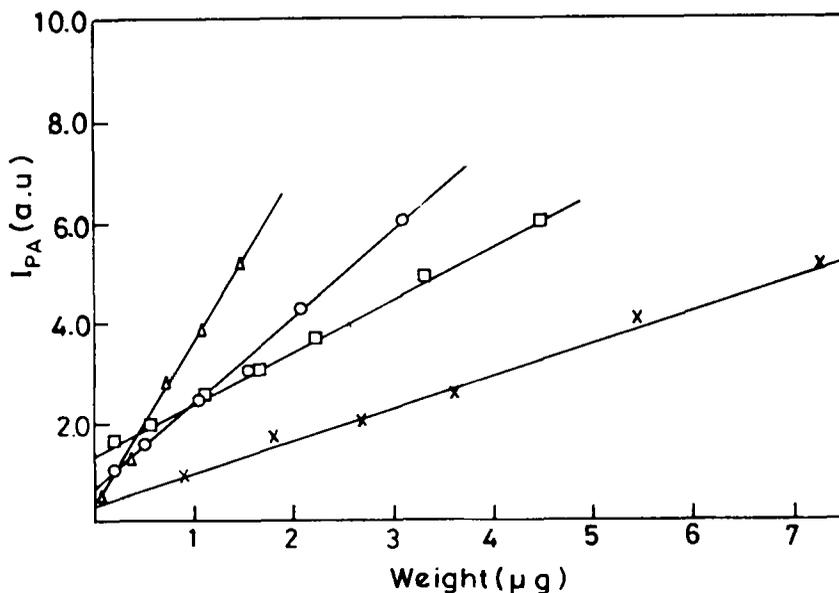


Figure 4. I_{PA} vs weight of various substances spotted on Whatman-41 filter paper at the absorption maxima of the unnormalized spectra. Circles, crystal violet (580 nm); triangles, neutral red (520 nm); squares, cytosine (280 nm); crosses, L-tyrosine (290 nm).

does not result in any artifacts. This indicates that one can correlate I_{PA} with concentration up to a concentration range. Normalized PA spectra shown in figure 2 resemble that of standard spectra of these substances showing that in the present case the spectra have not changed significantly even under conditions of adsorption. With a proper calibration curve we can, therefore, use PAS for the quantitative estimation of substances spotted on filter paper.

The nearly linear dependence obtained with Whatman-41 filter paper (figure 4) may be exploited to advantage in the sense that the number of calibration points is reduced. This technique will undoubtedly be of great value in biology where quantitative estimation of substances spotted on filter paper often poses difficulties. Another important feature is the minimum detectable amount of the substances spotted, which in our case is of the order of a few hundred nanograms. This can be gainfully used to estimate materials which are present in very small amounts and/or

The non-linear dependence of I_{PA} on wt. (figure 3) observed is likely to be due to particle size and light scattering effects. This has been discussed, in the case of powder, in some detail by Monahan and Nolle (1977) and recently by Monchalín *et al* (1984). Similar non-linear behaviour of the amount of light absorbed by paper containing various dyes has been pointed out by Foote (1939) and analysed by Van den Akker (1968). These authors have found, in the case of dyes adsorbed on papers, that with decreasing amounts of the dye the scattering increases. This may explain the non-linear part in the low concentration region. Since background contribution to the signal can vary with the size of the dye spot, one has to exercise caution while spotting. Spots, where weight of substance spotted is held constant but volume varied, did not show any difference in I_{PA} . One can clearly see

that only the solvent front advances, leaving the same sized spot in all the cases irrespective of the volume used. In our case where substances are spotted on filter paper, the absorbing chromophore is present as a thin layer on a substrate which is transparent to the radiation employed. Little is known about the generation of PA signals in such systems where the thermal properties of the substrate dominate the amplitude of the PA signal even though it is transparent to radiation (and hence inherently PA inactive). Systems such as these like solid catalysts, thin-layer chromatograms, substances adsorbed on filter papers have not been completely understood either from the theoretical or the experimental point of view. We are engaged at present in such studies.

The fraction of light absorbed seems to roughly show a logarithmic dependence on absorption coefficient, β . Melamed (1963) proposed a theory of scattering of light by particulate matter, from which the fraction of light absorption seems to show a logarithmic dependence on β for a considerable range of $\beta\delta$ ($10^{-3} \leq \beta\delta \leq 10^0$), where δ is the particle diameter. We do indeed find a nearly linear logarithmic dependence between I_{PA} and log wt. as shown in the inset of figure 3. This dependence could be usefully employed for the purpose of calibration and estimation.

Acknowledgements

Authors thank Professor C N R Rao, FRS, for suggesting the problem and useful discussions. One of us (TS) is thankful to the CSIR, New Delhi, for a fellowship.

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