THIN-LAYER CHROMATOGRAPHY ON SILICA GEL: QUANTITATIVE ANALYSIS BY DIRECT U.V. SPECTROPHOTOMETRY*

R. DONALD SPENCER AND BEVERLY H. BEGGS**
Mellon Institute, Pittsburgh, Pa. (U.S.A.)
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INTRODUCTION

An infrared spectrometric method has recently been developed in this laboratory[1] for the quantitative analysis of samples containing 2–3 mg of a mixture of triphenyl-*s*-triazine and its three *para*-chlorinated derivatives. However, an individual triazine cannot be determined by this method if it is present at a level of only a few percent in the mixture. Since these compounds absorb intensely in the ultraviolet (Table I), and since they are cleanly separated by chromatography on thin layers of silica gel (Fig. 1), they were chosen as a model with which to study the application of thin layer chromatography (TLC) to quantitative ultramicro spectrometric analysis.

The excellent resolving power, rapidity and simplicity of TLC makes it attractive in the analysis of mixtures that are too low in volatility or too thermally sensitive for the application of gas chromatographic techniques. A variety of quantitative methods built around TLC have sprung up in the decade since the pioneering work of KIRCHNER, MILLER AND RICE[2] in 1954. These have been summarized in several recent books[3–7].

Methods fall into two general categories: (1) measurement of spot area or optical density without removal of the sought-for substance from the plate, and (2) extraction of the substance from the adsorbent followed by analysis, usually by direct spectrometry or a colorimetric procedure***. Spot area measurement is most useful when the analyst has an intimate knowledge of the sample history, such as in routine quality control work, or when the amount of sample available is too limited for any other method. Direct spectrometry, which is the subject of this investigation, has the advantage that a U.V. or visible spectrum of the substance is readily obtained as part of the analysis. This provides important additional information as to its identity and purity.[9]

There are two main requirements which must be met if the extraction–spectrometric approach is to give accurate results. First, the sought-for substance must be completely extracted from the adsorbent or, if this is not possible, a known and reproducible fraction must be recovered. Furthermore, the substance should be obtained in the pure state.

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** Present address: 22 Valley Road, Princeton, N.J.

*** The first method is said to be simpler and faster[4,8] and the second, more accurate[5,8].

TABLE I

U.V. ABSORBANCE OF *s*-TRIAZINES IN METHANOL

	Sample number	*Pure triazine wt. (mg)*	*Molar concn. in methanol* ($\times 10^6$)[a]	*Absorbance (5 cm MeOH)*	*Molar absorptivity,* $\varepsilon_{max} \cdot 10^{-4}$	
2,4,6-Triphenyl-*s*-triazine (λ_{max} = 268 mμ)	1	15.63	2.01	0.647		6.44
	2	12.38	1.60[b]	0.516		6.45
	3	0.520	0.84[c]	0.258		6.14
	4	15.00	1.93	0.623		6.46
	5	0.875	2.83[c]	0.855		6.05
	6	16.19	2.08[f]	0.638	6.13	6.15
			2.08[f]	0.642	6.17	
	7	15.13	1.94[f]	0.626	6.44	6.35
			1.94[f]	0.609	6.26	
	8	15.30	1.97[f]	0.618	6.29	6.30
			1.97[f]	0.621	6.32	
Average and S.D.					$\bar{X} = 6.3 \cdot 10^4$, $s = 0.16 \cdot 10^4$	
2-(*p*-Chlorophenyl)-4,6-diphenyl-*s*-triazine (λ_{max} = 273 mμ)	1	11.99	1.39[b]	0.494		7.09
	2	1.785	1.30[d]	0.458		7.06
	3	15.76	1.82[f]	0.615	6.75	6.75
			1.82[f]	0.616	6.76	
	4	1.954	1.42[d]	0.468		6.59
	5	14.49	1.68[f]	0.590	7.04	7.09
			1.68[f]	0.599	7.15	
	6	14.73	1.70[f]	0.572	6.71	6.73
			1.70[f]	0.575	6.75	
Average and S.D.					$\bar{X} = 6.9 \cdot 10^4$, $s = 0.22 \cdot 10^4$	
2-Phenyl-4,6-(*p*-chlorophenyl)-*s*-triazine (λ_{max} = 277 mμ)	1	1.985	1.32[d]	0.465		7.05
	2	13.71	1.44[f]	0.517	7.18	7.13
			1.44[f]	0.511	7.09	
Average						$7.1 \cdot 10^4$
2,4,6-Tri(*p*-chlorophenyl)-*s*-triazine (λ_{max} = 283 mμ)	1	1.945	1.18[e]	0.430		7.30
	2	8.77	0.845[f]	0.279	6.60	6.93
			1.69[f]	0.614	7.27	
	3	9.96	1.92[f]	0.739	7.70	7.59
			1.92[f]	0.717	7.47	
	4	8.57	1.65[f]	0.641	7.76	7.70
			1.65[f]	0.631	7.64	
	5	8.38	1.61	0.619		7.67
Average and S.D.					$\bar{X} = 7.4 \cdot 10^4$, $s = 0.33 \cdot 10^4$	

[a] Except where otherwise noted, a stock solution was made by dissolving the pure triazine in carbon tetrachloride in a 25-ml volumetric flask. A 49.7 μl sample was transferred by means of a calibrated micro syringe to a 50-ml volumetric flask, the solvent was evaporated, and the residue was dissolved and brought to the mark with reagent grade (low U.V. absorbance) methanol.

[b] The sample was dissolved in methanol in a 500-ml volumetric flask and a 5-ml aliquot was further diluted to 250 ml.

[c] The sample was weighed on a micro balance and dissolved directly in methanol.

[d] The sample was weighed on a micro balance, dissolved in methanol, and brought to one liter. A 25-ml aliquot was diluted to 100 ml.

[e] This was similar to (d), but the sample was made up to two liters and a 50-ml aliquot was diluted to 100 ml.

[f] Samples were treated as in (a), but duplicate 49.7 μl aliquots were taken from the same stock solution.

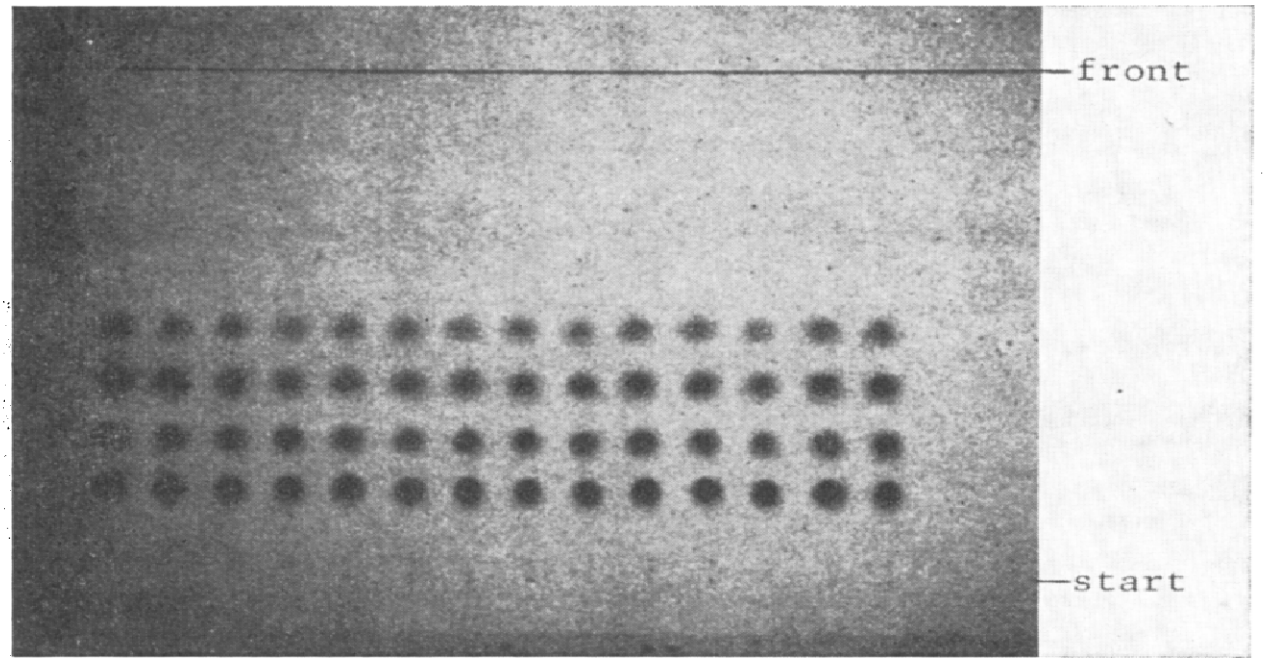

Fig. 1. Separation of triaryl *s*-triazines on silica gel. Top row: 2,4,6-tri(*p*-chlorophenyl)-*s*-triazine; second row: 2-phenyl-4,6-(*p*-chlorophenyl)-*s*-triazine; third row: 2-(*p*-chlorophenyl)-4,6-diphenyl-*s*-triazine; bottom row: 2,4,6-triphenyl-*s*-triazine. Chromatography on 250 μ layer of Brinkmann Silica Gel GF with CCl_4 developing solvent. Photographed under a 9-watt "Mineralight" (Ultraviolet Products, Inc., South Pasadena, Calif., Model SL 2537) with maximum transmission near 254 mμ (short wave U.V.).

These requirements have not been adequately fulfilled in most of the methods that have been reported in detail in the literature. Seldom have authors claimed complete recovery of compounds from TLC plates. Moreover, the reasons for the losses do not appear to be well understood. Similarly, it must be deduced that analytical blanks have generally been high and unreproducible (although specific absorbance data have rarely been reported in the literature). The reason for the existence of these substantial blanks has not been satisfactorily explained.

Recovery of compounds from TLC plates

KIRCHNER, MILLER AND RICE[2] and many who followed them[9–14], have begged the question as to whether or not the compounds being analyzed were completely extracted from the silica gel before spectrometric determination was carried out. KIRCHNER and his co-workers[2] avoided this issue by running standard solutions of the sought-for substance (biphenyl) through their procedure, constructing standard curves, and using these to establish the biphenyl content of unknown samples.

Among the other authors who chose a similar approach, STRUCK does claim[12] 100 % recovery of estrogens from silica gel by extraction with 0.5 *N* sodium hydroxide in 80 % ethanol, but he offers no supporting data for the statement.

Other authors[15–19], while still using empirical approaches similar to those mentioned above, have also investigated the question of how completely the substances being analyzed are recovered from the TLC plates. The losses due to incomplete extraction of compounds from the silica gel adsorbent, even when recovery was attempted before the plate was developed (that is, the substance was applied and the

silica gel containing it was scraped off and extracted), have been in the range 4–8 % for esters of *p*-hydroxybenzoic acid[15] and steroids[18], and as high as 20–25 % for three cardiac glycosides from squill[17].

Even greater losses have resulted when the compounds were allowed to traverse the plate during chromatographic development before they were extracted from the silica gel[15,17]. GÄNSHIRT, KOSS AND MORIANZ[16] have reported rather wide deviations on both sides of 100 %, when nine bile acids were individually chromatographed on silica gel layers, extracted with 65 % sulfuric acid, and warmed at 60° to develop maximum absorbance at the analytical wave lengths near 385 mμ. Sample absorbances ranged from about 70 to 120 % of those obtained when the compounds were treated directly with the sulfuric acid reagent. The authors stated that since the chromatographed samples gave reproducible absorbances* the discrepancies were due to the influence of silica gel.

The loss of about 2.5 % of a chromatographed steroid upon extraction from silica gel, reported by BIRD and co-workers[19], may not really be significant.

GÄNSHIRT in STAHL's book** reports that hydrocortisone alcohol and acetate can be completely recovered from Silica Gel G by extraction with ethanol.

SCHILCHER[20] has recently described a "vacuum cleaner" device which transfers the adsorbent containing the separated substance directly from the plate to a centrifuge tube containing the extracting solvent. He claims good results in the quantitative analysis of various biological substances, including vitamin D_2.

MILLETT, MOORE AND SAEMAN[21] report complete recovery of furoic acid and almost complete recovery of hydroxymethylfuroic acid from silica gel layers.

Factors relating to the silica gel blanks

Silica gel is, without doubt, the most versatile adsorbent in the quantitative analysis of compounds separated on thin layer chromatographic plates. This is because the resolving efficiency of this adsorbent is generally good, and most compounds can easily be recovered from it by extraction with polar solvents such as methanol. However, its use for spectrometric analysis in the ultraviolet region has been attended by difficulties with high and inconsistent blanks. This problem has been attacked in many ways. The silica gel has either been extracted in powder form[12,15,18] or after being made up into a layer[2,10].

HONEGGER, in his study of preparative aspects of TLC[22], found it necessary to pre-purify both the silica gel and the solvents used in the extraction of the chromatographed constituents, when quantitative analysis was to be carried out. He obtained, for example, 12 mg of non-volatile residue (unidentified) by eluting 50 g of Silica Gel G with 200 ml of acetone, of which 1.5 mg was contributed by the solvent and the remainder by the silica gel.

When pre-extraction of the silica gel is not practiced, most workers have considered it necessary to make blank determinations on areas of silica gel from the same plate as the sample[16,19]. SCHLEMMER AND LINK[11] took an area of silica gel equal to the sample spot at the same R_F location on a second, blank plate, which they developed simultaneously with the sample plate.

* The method did not prove as reproducible in the hands of HOFMANN (A. F. HOFMANN in ref. 8, p. 280).

** Ref. 3, English ed., Table IV, p. 53.

Even when pre-extraction of the silica gel *has* been carried out, it has been considered advisable to run blanks. KIRCHNER[2] and STANLEY[10] and their co-workers ran several blanks at the start of each working day. Later workers preferred to take their blanks from the plates on which the samples had been chromatographed[12,15].

The actual absorbances of silica gel blanks have rarely been reported in the literature, undoubtedly because the general use of double beam spectrophotometers permits the blanks to be subtracted optically. However, an idea of the variability which can be expected may be deduced from the fact that several workers took care to remove for their blanks areas equal in size to those occupied by the sample spots and at locations on the plate with precisely equivalent R_F values[11,12,15,16,19].

The nature of the impurities in the silica gel has apparently not been established. One commercial supplier of silica gel adsorbents for TLC claims that other brands on the market contain impurities which are revealed by (1) background grayness when plates are sprayed with sulfuric acid and heated, and (2) methanol-extractable material which gives a number of peaks when the solvent is concentrated and analyzed by gas chromatography[23,24].

GÄNSHIRT AND MORIANZ[15] attempted to reduce their silica gel blanks by extracting the powder with methanol for 8 h before making up the plates. This reduced the blank to about half its original value at 225 mμ; at wavelengths above 240 mμ the improvement was much smaller. The extracted impurities were not identified*. TRUTER, in reviewing this and other similar studies**, concludes that "impurities which absorb ultra-violet energy are extracted from the adsorbent", but he draws no conclusions as to their nature.

EXPERIMENTAL

Equipment

Spectra. U.V. spectra were determined with a Cary Model 14 recording spectrophotometer. Particular care was taken in positioning the cells and calibrating the instrument. Absorbance reproducibility was usually better than ± 0.002 absorbance units claimed by the manufacturer.

Silica gel. Merck Silica Gel GF_{254} was used (distributor: Brinkmann Instruments Inc.).

TLC plates. Glass plates 200 × 200 mm were coated with silica gel slurry, with the spreader set at a thickness of 250 microns (the dry layer is thinner***).

Scraper. A convenient scraper was made by cutting off a 16-mm wide stainless steel spatula. This permitted a clean and rapid transfer of a 16-mm wide swath of silica gel from a plate to a weighing paper.

Membrane filters. Most of the work was done with "Polypore AM-6", a product of the Gelman Instrument Company. The manufacturer describes it as a "cellulose ester"§. This filter must be kept wet. It no longer appears to be on the market, but has

* GÄNSHIRT later suggests[3] (English ed., pp. 52–53) that "unexpectedly high and scattered blank values... may be due to incomplete removal of UV-absorbing impurities in the solvent". He also states that peptization of the silica gel adsorbent by hydrophilic solvents can occur, but, in many cases, the silica gel can be removed by filtration through a short kieselguhr column.

** Ref. 4, p. 105.

*** E. STAHL[3], English ed., p. 27.

§ It is apparently the triacetate, since this is the only cellulose ester which shows excellent resistance to methanol[25].

been replaced by other cellulosics[25]: "Metricel-GM" (cellulose acetate), "Metricel-GA" (cellulose triacetate), and "Alpha Metricel" (regenerated cellulose). The last type has been used in this laboratory in a few experiments and appears to be satisfactory. S and S membrane filters No. 01 and 02 also appear to be satisfactory. All filters were washed well with methanol before use, to extract a small amount of U.V.-absorbing, methanol-soluble material.

Methanol. "Baker Analyzed" reagent grade methanol (not spectro grade) was used, but each newly opened bottle was tested first for absorption in the ultraviolet. (Some lots from another manufacturer were found to absorb strongly below 300 mμ.) Filtration of bottled reagent methanol through well-washed filters resulted in a slight but consistent decrease in absorbance, which indicates that a small amount of filterable material can be removed from the solvent.

Absorbance vs. concentration of s-triazines

The U.V. spectra of standard solutions of pure *s*-triazines in methanol were determined in 5-cm quartz cells. Peaks were symmetrical and had strong maxima near 275 mμ. Table I indicates that, within the limits of accuracy of the measurements, Beer's law is obeyed up to a concentration at least as high as 2.0×10^{-6} *M*.

s-Triazines on silica gel layers

A volume of 49.7 μl of the *s*-triazine solution was applied as a row of spots across a 200 × 200 mm plate, by means of a 50 μl Hamilton gas-tight syringe which had been calibrated with mercury. Twice this volume was used for the more dilute tri(*p*-chlorophenyl)-*s*-triazine solutions.

Triazines were recovered from silica gel layers and determined spectrometrically in the manner described in the following paragraphs. Concentrations of the methanol solutions were calculated from the absorbances by means of Beer's law, with molar absorptivities taken from Table I.

Removal of samples and blanks from TLC plates

The scraper was used to cleanly remove a 16-mm swath (about 0.28 g) of silica gel from a 200 × 200-mm plate horizontal to the direction of development, after first scraping off and discarding about 1 cm from the left and right edges (which contained no sample and which were in an area most likely to be contaminated by handling). The powder was transferred by means of weighing paper to a membrane filter mounted in the holder described below.

Membrane filtration and measurement of U.V. absorption

An all-glass funnel was constructed which consisted of an upper tubular member and a lower funnel, between which a membrane filter could be clamped. The surfaces holding the membrane were flat and finely ground, to prevent leakage during filtration, and the lower member contained a coarse-porosity sintered disk (24 mm diameter) in contact with the membrane, to provide support and prevent its distortion during filtration. With the membrane in place, approximately 100 ml of methanol was run slowly through the funnel to insure removal of all methanol extractable substances from the membrane before adding the silica gel sample.

The silica gel powder was poured on to the wet membrane and extracted with

TABLE II

ABSORBANCE OF SILICA GEL POWDER BLANKS[a]

Treatment	Absorbance			
	At 270 mμ		At 400 mμ	
	Trials	Av.	Trials	Av.
Decantation				
Supernatant taken by syringe after overnight settling	0.156 0.433	0.29	0.062 0.347	0.20
Cotton plug				
Through a tight cotton plug	0.126 0.137	0.132	0.022 0.033	0.028
Fritted glass				
Coarse frit, 40–60 μ[b]	0.345[c] 0.152	0.25	0.248 0.128	0.19
Fine frit, 4–5.5 μ[b]	0.070 0.072	0.071	0.047 0.015	0.031
Ultra-fine frit, 0.9–1.4 μ[b]	0.050 0.030 0.019	0.033	0.004 0.008 0.001	0.004
Centrifugation[d]				
3,000 r.p.m. for 5 min	0.077[e] 0.061[f]	0.069	0.040 0.025	0.032
10,000 r.p.m. for 30 min	0.020 0.024	0.022	0.004 0.004	0.004
Membrane filtration[g]				
Gelman "Polypore" 27 A, 0.45 μ	0.025 0.024	0.024	0.008 0.008	0.008
S and S 01, 0.4 μ	0.020 0.023	0.022	0.003 0	0.002
Gelman "Polypore" 27 E, 0.03 μ	0.020 0.017	0.018	0.003 0.003	0.003

[a] Brinkmann Silica Gel GF (which contains $CaSO_4$ binder and a manganese-activated zinc silicate fluorescent indicator) was used directly from the bottle, 0.28 g per trial. This amount is equivalent to a 16 mm swath across a 0.25 mm (spreader setting) layer on a 20 × 20 cm plate. Sufficient methanol was used (almost always 50 ml) to fill a 5 cm quartz cell.

[b] Maximum pore diameter ratings in Corning Glass Works, LG-3 Catalog, p. 216 (1963).

[c] Filtration of this solution through a Gelman "Polypore" 27 A (0.45 μ) membrane reduced this absorbance to 0.023.

[d] Samples were carefully taken by means of a clean syringe without removing tubes from the centrifuge.

[e] Filtration of this solution through a Gelman "Polypore" 27 A (0.45 μ) membrane reduced this absorbance to 0.033.

[f] Filtration of this solution through a Gelman "Polypore" 27 A (0.45 μ) membrane reduced this absorbance to 0.022.

[g] Membranes were clamped between ground glass faces in a glass funnel and supported by a coarse frit sealed into the lower member. Filtration was by gravity flow in these tests, and membranes were pre-rinsed with about 100 ml of methanol to insure removal of all membrane extractibles.

TABLE III

ABSORBANCE OF SILICA GEL BLANKS* SCRAPED FROM TLC PLATES USED TO CHROMATOGRAPH TRIPHENYL-*s*-TRIAZINE

(5 cm, methanol)

	*Absorbance of silica gel blanks**		
	(*a*) 270 mμ	(*b*) 400 mμ	(*a*) — (*b*)
Plate No. 1			
Undeveloped area	0.017	0.005	0.012
Developed area (top)	0.013	0.001	0.012
Developed area (bottom)	0.012	0	0.012
Plate No. 2			
Undeveloped area	0.013	0	0.013
Developed area (top)	0.016	0.003	0.013
Developed area (bottom)	0.018	0.005	0.013

* The silica gel in these plates was from a freshly opened bottle.

TABLE IV

RECOVERY OF TRIAZINES FROM SILICA GEL LAYERS[a]

	Absorbance			*Percent recovery*
	Sample	*Blank(s)*	*Net*	
Triphenyl-s-triazine[b]				
Solution determined directly[c]	0.632		0.623	100
Spotted on plate but not developed	0.608	0.017[d]	0.591	94
	0.608	0.013[d]	0.595	94
Spotted on plate and developed	0.570	0.013, 0.011[e]	0.558	88
	0.591	0.016, 0.018[e]	0.574	91
Tri(p-chlorophenyl)-s-triazine[f]				
Solution determined directly[g]	0.619		0.619	104
Spotted on plate but not developed	0.610	0.020[d]	0.590	99
	0.600	0.019[d]	0.581	97
Spotted on plate and developed	0.586	0.021, 0.022[e]	0.564	94
	0.614	0.026, 0.027[e]	0.587	98

[a] Two 200 × 200 mm plates used for each triazine.

[b] Triphenyl-*s*-triazine stock solution: 0.01559 g in CCl_4 (25-ml volumetric flask).

[c] Aliquot (49.7 μl) of CCl_4 solution evaporated and residue dissolved in methanol (50-ml volumetric flask).

[d] One equally wide blank scraped from the undeveloped part of the plate.

[e] Two blanks of equal width scraped from the developed part of the plate, one above and the other below the sample area.

[f] Tri(*p*-chlorophenyl)-*s*-triazine stock solution: 0.00838 g in CCl_4 (25-ml volumetric flask).

[g] Two 49.7-μl aliquots of CCl_4 stock solution combined, evaporated, and residue dissolved in methanol (50-ml volumetric flask).

40 ml of methanol, which was ordinarily allowed to flow through by gravity. The filtrate was caught in a 50-ml volumetric flask and was diluted to the mark with methanol.

Unless otherwise stated, absorbances were measured by means of 5-cm quartz cells, with methanol in the reference cell (from the same bottle as was used to extract the samples).

Results are summarized in Tables II, III and IV. When *net* absorbance was desired (Table IV), blanks were run on equal-sized strips taken parallel to the sample strips and from the same plates.

DISCUSSION OF RESULTS

Laboratory contaminants

Early attempts to recover the triazines from chromatographic plates were inconclusive, largely because of high and variable blanks. Although the magnitude of the blanks might readily be attributed to soluble impurities extracted from the silica gel itself, it did not seem reasonable that this cause could also explain the lack of consistency in the results when only one bottle of silica gel was being used.

Laboratory atmospheric pollutants were suspected of causing contamination of the plates, since such impurities are abundant and varied in our laboratory. Indeed, exposure of a plate to a high concentration of cigarette smoke resulted in the adsorption of a methanol-extractable material, which absorbed strongly in the same region of the ultraviolet in which the triazine peaks are found (Fig. 2). Also, on one occasion a

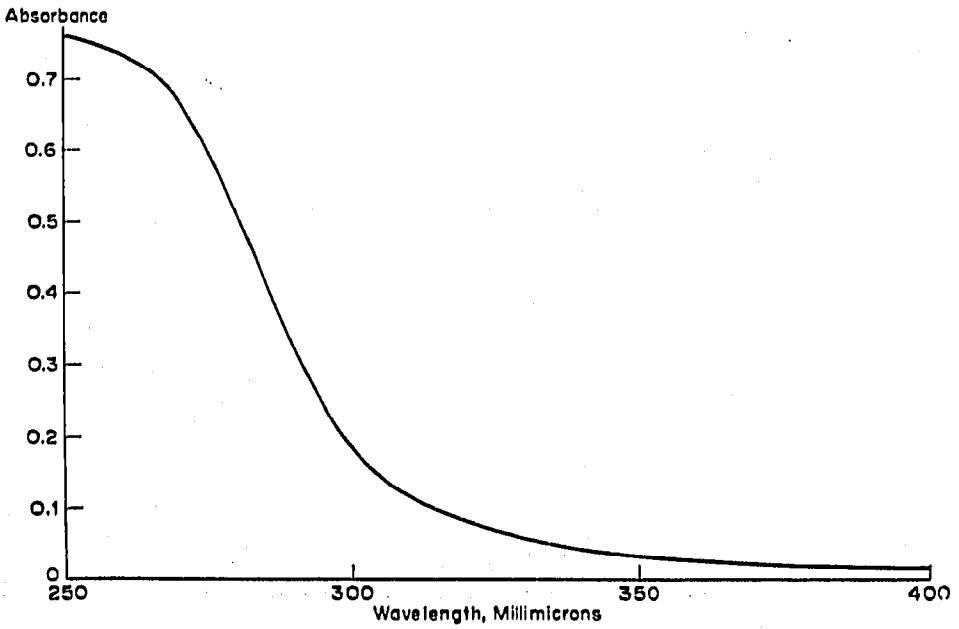

Fig. 2. Cigarette smoke eluted from TLC plate (1 cm, methanol).

sprinkling of tiny dark spots was observed when the fluorescent plates were viewed under short-wave-length U.V. light and the spots were found to migrate when the plates were developed with carbon tetrachloride. The same effect was obtained when a plate was very lightly sprayed with a light lubricating oil. It was therefore suspected

that the contaminant in this case had been an oily "fall-out" from the ventilating system.

Finely divided sooty material, known to be present in substantial amounts in the ventilator air, was also believed to have a large effect on the blanks.

Concern with laboratory contaminants such as the examples described above occupied a considerable amount of time, and led down many blind alleys. In order to avoid such contamination, plates were prepared in as clean an area as possible and great care was exercised in the cleaning and handling of glassware. Once, a series of experiments was run with as many manipulations as possible carried out in a dry box flushed with filtered and purified air. However, blanks were still high and inconsistent.

Solvents

Two solvents, carbon tetrachloride and methanol, as well as mixtures of these two, were investigated in the elution and spectrometric determination of the triazines. Although these compounds are much more soluble in carbon tetrachloride than in methanol[1], the former solvent was rejected because it starts to absorb strongly itself in the vicinity of the triazine peaks, and because it is not very efficient in extracting triaryl triazines from silica gel. Addition of some methanol improved eluting efficiency but introduced uncertainty as to solvent composition of the final solutions, thereby making it difficult to determine what solvent mixture to use in the reference beam of the spectrophotometer.

Pure methanol has excellent transparency in the vicinity of 275 mμ and effectively elutes the triazines from silica gel, but these compounds, particularly 2,4,6-tri(*p*-chlorophenyl)-*s*-triazine are very sparingly soluble in this solvent[1]. Nevertheless, methanol was picked as the solvent of choice, and the necessarily high dilution of the solutions was compensated for by the use of 5-cm rather than 1-cm cells.

The problem of high and inconsistent blanks, however, was proportionately increased, and discrepancies which might have been small enough to be considered acceptable at short path lengths, with more concentrated samples, became unacceptably large under these more stringent conditions.

Preparation of silica gel blanks

Silica gel scraped from the plates for blank or sample determination was eluted in a variety of ways. The simplest approach was filtration through cotton, such as was used by BIRD *et al.*[10]. The powder was poured on to a plug of methanol-rinsed cotton in a glass tube drawn out to a medicine-dropper tip and extracted with methanol. However, this procedure gave a high blank, and it was thought that some ultraviolet-absorbing impurities were continuing to be extracted from the cotton itself.

Pyrex glass wool was therefore substituted, but blanks of silica gel suspensions filtered in this manner were no better. Furthermore, surprisingly, the pre-extracted glass wool itself was found to have a measurable blank when it was stirred with solvent in the absence of any silica gel. Not only that, but the absorbance of these blanks continued without much reduction in strength as the wavelength was increased from 300 mμ to 400 mμ (in the visible region).

Filtration of the silica gel slurry through coarse fritted glass also gave high blanks. Use of diatomaceous earth filter aid, as suggested by GÄNSHIRT*, was not

* STAHL[3], English ed., p. 52.

satisfactory. It was observed at this time that filtrates from silica gel slurries, while appearing clear by transmitted light, exhibited a definite Tyndall scattering effect. This was also true with pure methanol that had been passed through filter aid alone, so the use of this material was discontinued.

It started to become apparent at this point that, whereas triazines dissolved directly in a pure solvent almost never absorbed at 400 mμ or above, solutions obtained by elution of the compounds from silica gel almost always showed some absorption at 400 mμ. Fig. 3 shows absorption curves of identical amounts of triphenyl-*s*-tria-

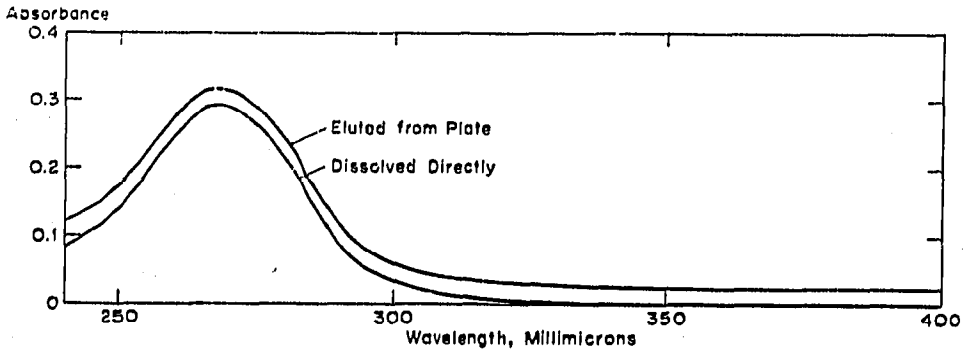

Fig. 3. U.V. absorbance of triphenyl-*s*-triazine (1 cm, methanol). Spectra are from 50 μl of $2.26 \cdot 10^{-3}$ *M* CCl_4 stock solution. Residue (free from CCl_4) was taken up in 25 ml methanol and measured in a 1-cm cell against pure methanol. The solution of sample recovered from silica gel was filtered through hardened filter paper.

zine, one sample dissolved directly in methanol and the other spotted on a silica gel plate and recovered from the adsorbent with methanol. Filtration in this case was through hardened filter paper. Comparison of the two curves brings out the further fact that the surplus absorption at 400 mμ often carries through the entire upper U.V. spectrum in a surprisingly uniform manner. Thus, absorption of the eluted solution, the upper curve, is too high at the triazine maximum near 270 mμ by approximately the same amount that this solution absorbs at 400 mμ. The strength of absorption at 400 mμ was subject to wide fluctuation from sample to sample.

Fig. 4 shows the type of absorption curves obtained from a variety of particulate materials. "Ventilator dirt" was sooty dust which had been carried into the laboratory by a stream of air from a partially blocked ventilator and deposited on the wall. A suspension of this was made up in methanol, allowed to settle and decanted into a cell. The cellulose fibers were obtained by pulling apart a paper tissue. The two middle curves represent Brinkmann Silica Gel GF taken directly from the bottle, suspended in methanol, and filtered through new fritted funnels which had previously been thoroughly rinsed with the same solvent. The structureless, almost constant absorption of all these suspended solids is noteworthy.

It is clear that lint and atmospheric dust could hardly be expected to contribute seriously to the high blanks that have been reported in the past, since accidental contamination by these materials could lead to only a very small fraction of the absorbances shown in the figure.

On the other hand, finely divided silica gel, incompletely removed by filtration, could easily account for the major portion of the absorption observed in the blanks.

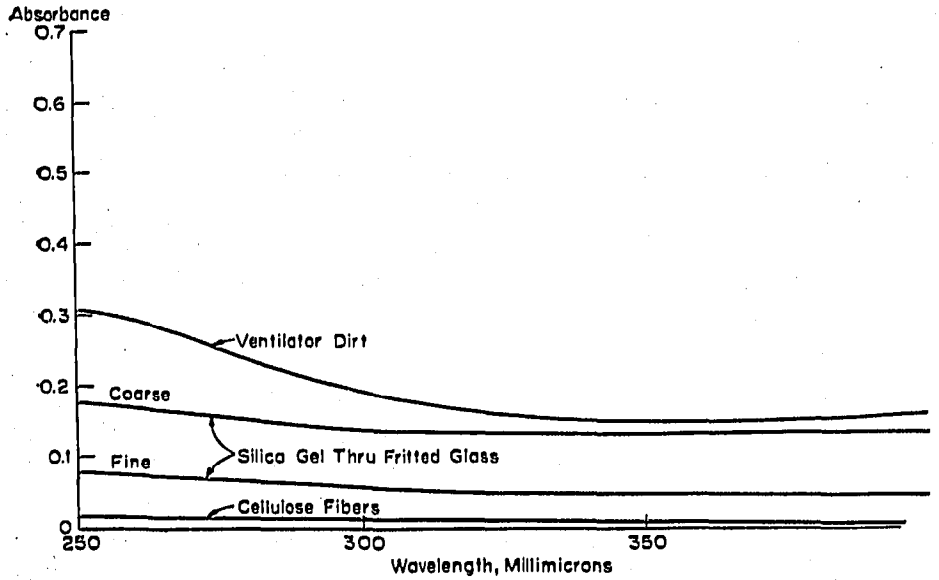

Fig. 4. Spectra of "dirt" (5 cm, methanol).

The solvent of choice, methanol, leads to particularly high blanks because of the very large difference in refractive index between it and silica gel.

The clue that particulate material was responsible for much of the absorption of silica gel blanks was followed up by subjecting suspensions of silica gel to filtration through finer and finer filters. It is clear from Table II that one must go to glass frits of ultra-fine porosity before one can be confident of having removed most of the offending particles. Frits of this porosity filter extremely slowly, are easily plugged, and are virtually impossible to clean properly.

Ordinary centrifugation as practiced by some authors[11–13] is not adequate*. Thirty minutes at 10,000 r.p.m., however, does appear to be satisfactory**.

The most convenient method of removing finely divided solids, however, is by filtration through synthetic membrane filters with pore size of about 0.4 μ. Membranes are now available which are completely resistant to methanol and most other organic solvents[25]. Filtration is rapid, even without use of pressure or suction, and filters can economically be discarded after each use. Consistently low blanks can be obtained by this approach for 5 cm path lengths, and 10 cm path length might reasonably be considered with transparent solvents such as methanol.

The fact that low blanks are obtained when finely divided insoluble matter is removed provides strong evidence that the ultraviolet absorbers which have previously

* GÄNSHIRT, KOSS AND MORIANZ[16] do not state the speed of centrifugation. SCHILCHER[20] states only that the sample was centrifuged vigorously for some time and for greater purity, cautiously decanted through a sintered glass "G4" filter (medium porosity).

** Other methods of clarifying extracts of silica gel for direct U.V. spectrometric analysis that have been reported are filtration through cotton[10], a thin asbestos mat[21,26], or sintered glass of medium[14,15,18], or unspecified porosity[2,9,10]. The "vacuum cleaner" technique, in which the adsorbent is collected on a glass frit or some other porous surface where it can subsequently be extracted, has been used frequently in the past[18,19], and is becoming increasingly popular[20,21,27]. Filters suitable for this purpose should have a pore size no smaller than 20–25 μ[28], which is not fine enough to filter out fine particles of adsorbent. Furthermore, it should be noted that many compounds which are relatively stable in air are *not* stable when they are spread over the large area of the adsorbent surface (GÄNSHIRT[3], English ed., p. 52, and HONEGGER[22], p. 1413), particularly in the dry state[29].

been assumed to be impurities present in the solvents or silica gel are, primarily, finely divided silica gel itself. This, however, does not mean that small amounts of soluble impurities may not also be present in some samples of the adsorbent. As mentioned above, silica gel takes up many organic vapors rapidly from the air. The blanks obtained after filtration of slurries of Silica Gel GF in methanol through 0.45μ membranes were reduced substantially when the silica gel was taken from a freshly opened bottle (compare Tables II and III). The old bottle (data of Table II) had been used a long time and its contents had frequently been exposed to the atmosphere.

While many silica gel slurries filtered through 0.45 μ membranes have had zero absorption at 400 mμ with reference to pure solvent, this is by no means universally true, as can be seen from Table III. This data was obtained by developing two blank plates in carbon tetrachloride, scraping off one strip of silica gel in the upper, undeveloped area and two others near the top and bottom of the developed area. Each adsorbent sample was eluted with methanol on a fresh 0.45 μ "Polypore AM-6" membrane. The blanks differed slightly from each other at 270 mμ (where the triphenyl-*s*-triazine peak is found), and most of them also were found to absorb slightly at 400 mμ. The observation that subtraction of the absorbances at 400 mμ from those at 268 mμ reduces the scatter and gives closely agreeing values leads one to suspect that absorbance at 400 mμ in these samples is an indication that not all the silica gel was being removed by the filter.

Table III indicates that there was no significant difference in the size of blanks taken from different empty regions of the same developed (CCl_4) plate. Thus, in our work there was no need to take the blank at the same mobility (R_F value) as the sample spot. It should be stressed, however, that blanks would not be expected to be equivalent at all distances from the start if the silica gel contains significant amounts of soluble U.V.-absorbing impurities or if the finished plate has been exposed for any length of time to a contaminating atmosphere. The polarity of the developing solvent is also an important factor: impurities not moved by carbon tetrachloride might be moved by a more polar solvent such as chloroform.

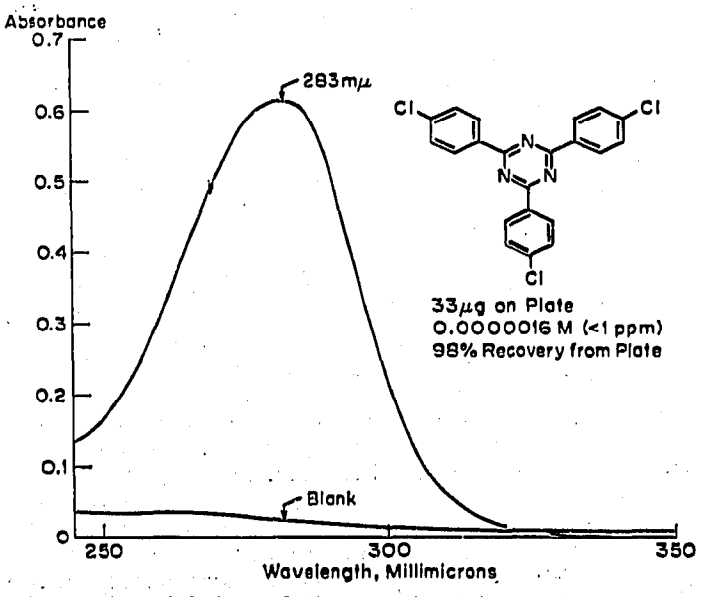

Fig. 5. Sensitivity of the method (5 cm, methanol).

Only a few experiments involving the actual recovery of triazines from thin layers of silica gel were performed subsequent to developing satisfactory filtration techniques. Table IV shows some of these results. Triazine recovery from silica gel does not appear to be complete despite the fact that it moves very readily on the thin layers, even with a weakly polar solvent like carbon tetrachloride. It is not likely that manipulation losses can account for this discrepancy.

Fig. 5 is the absorption curve of one of the samples given in Table IV, which illustrates the potential sensitivity of the method when the problem of high and unpredictable blanks is avoided by ultrafiltration. Only 33 μg of tri(*p*-chlorophenyl)-*s*-triazine was applied to the plate. The chromatogram was developed with carbon tetrachloride and 98 % of the original triazine extracted from the silica gel with 50 ml of methanol. The concentration of this methanol solution was 1.6×10^{-6} *M*. It should be noted that sensitivity might be increased severalfold by using less methanol in the extraction and making the U.V. absorption measurements in long-path micro cells. The blank has only a few percent of the absorbance of the sample but in this particular case the blank continues to absorb slightly up into the visible. It is believed that this absorbance is due to finely dispersed particles that were not filtered out by the membrane filter.

SUMMARY

The presence of soluble impurities[22,23] in silica gel, which could not be completely removed by pre-extraction of the powder itself[12,15] or of the prepared plates[2,10], has hampered the development of methods for the quantitative analysis by direct ultraviolet spectrometry of substances separated by thin-layer chromatographic techniques. Blanks have generally been high and inconsistent*. A large fraction of the absorbance in the ultraviolet of methanolic extracts of the adsorbent can be eliminated by filtration through a 0.45 μ synthetic membrane filter, which suggests that the "impurity" in question is probably finely dispersed silica gel itself. Filtration of methanol suspensions of an ordinary commercial silica gel (0.28 g of Brinkmann Silica Gel GF in 50 ml final volume of solvent) gave absorbances of 0.015 to 0.025 at 270 mμ and a 5 cm path length.

Preliminary experiments indicate the feasibility of ultramicro quantitative analysis of triaryl-*s*-triazines separated on thin-layer chromatographic plates.

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* MATTHEWS, PEREDA AND AGUILERA[18] report that "erratic or high results" were avoided when the adsorbent was pre-extracted with methanol. Their procedure resulted in removal of fines as well as soluble impurities.

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