

## THE QUANTITATIVE SEPARATION OF TETRAPHENYLPORPHINES BY THIN-LAYER CHROMATOGRAPHY

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Previous work demonstrated that tetraphenylporphines (TPP) are formed in the course of ultraviolet irradiation of mixtures of benzaldehyde, pyrrole and water<sup>1,2</sup>. The isolation of TPP from other pyrrole containing compounds formed was effected utilizing a modification of the column chromatographic method of PRIESTHOFF AND BANKS<sup>3</sup>.

The need arose for a method of isolation of TPP which was less time consuming, more sensitive, and more reproducible.

Thin-layer chromatography has been used successfully for the separation of the naturally occurring coproporphyrins I and III<sup>4</sup> and of various porphyrin esters<sup>5</sup>.

The method described herein represents the application of thin-layer chromatography to the rapid, quantitative isolation of TPP from a complex mixture of pyrrole-containing compounds.

## EXPERIMENTAL

All chromatographic plates used in this method were of ordinary type of glass measuring 20.0 × 5.0 cm. Camag Kiesel Gel DO adsorbent is spread in uniform layers of 20  $\mu$  thickness from a slurry of 30 g of gel in 70 ml of 50 % ethanol using the Shandon Unoplan spreader. After spreading, the plates are air dried for approximately one-half hour, then activated for at least 1 h at 115°. After cooling, 25  $\mu$ l of the sample are applied at about 1.5 cm from the bottom of each plate using a 50  $\mu$ l or 100  $\mu$ l microsyringe. The plates are developed in a Shandon T.L.C. Chromatank charged with 1 % acetone in benzene (v/v) for a period of about 2 h, *i.e.* until the front travels at least 18.5 cm. The atmosphere in the tank is equilibrated with the developing solvent for at least 1 h before the plates are inserted. After the development of the plates, they are removed from the tank and air dried. The tetraphenylporphine band is located by illuminating the plate with 2537 Å mercury line light (Mineralight). The silica containing the band is scraped off with a microspatula and placed in the stem of a 9 cm long stem funnel, plugged with cotton at the bottom. The elution of this column is accomplished with pure benzene until 3 ml of effluent are collected.

The spectra of the eluted substance are recorded on the Bausch and Lomb, Spectronic 505 recording spectrophotometer in the range from 350 to 650 m $\mu$ . Bands at 418 m $\mu$  (Soret band) or 515 m $\mu$  are used for quantitative determination of the tetraphenylporphine. For quantitative estimation at the 515 m $\mu$  maximum, a molar

extinction coefficient of  $18.7 \cdot 10^3$  and at the Soret band, *i.e.*  $418 \text{ m}\mu$ , one of  $478 \cdot 10^3$  for the free base were used as suggested by THOMAS AND MARTELL<sup>6</sup>.

The standard tetraphenylporphine was prepared by the method of BALL, DOROUGH AND CALVIN<sup>7</sup> and its spectrum agreed with those published in the literature<sup>6</sup>

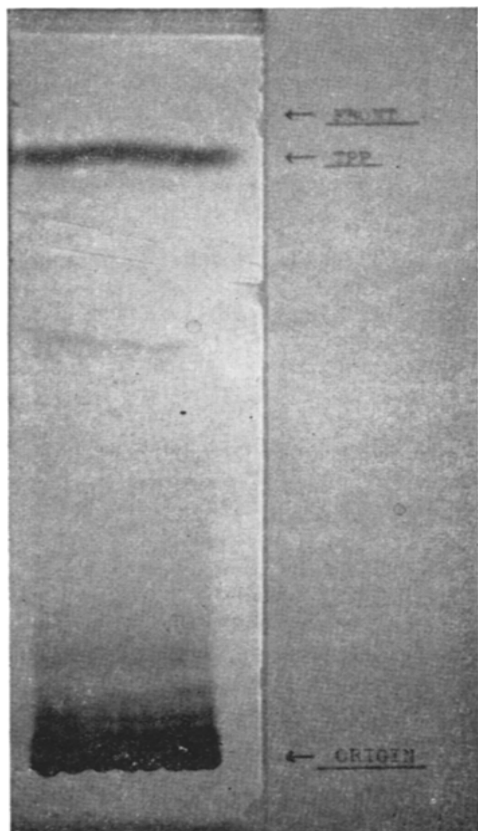


Fig. 1. The thin-layer chromatographic separation of TPP.

## RESULTS

Having found the solvent system which would separate TPP from all other compounds of our experimental solutions (Fig. 1) we tested those factors inherent in the overall method which were potential sources of error in the reproducibility and/or accuracy of the determinations.

In order to evaluate the reproducibility of measurement of  $25 \mu\text{l}$  of the solution using a  $100 \mu\text{l}$  microsyringe, 18 samples of standard TPP solution were measured and dissolved directly in 3.0 ml of benzene. The average of the absorbancies of these solutions at  $515 \text{ m}\mu$  was found to be 0.240 with a standard deviation of 0.013 units. Upon calculation it was found that each solution contained  $23.7 \mu\text{g}$  of TPP (Table I-A).

In all subsequent experiments wherein the efficiency of recovery is in question the recovery of the experimental, chromatographed sample is compared with a "standard" sample, *i.e.*, the same volume of TPP solution dissolved directly in 3.0 ml of benzene.

The quantitative recovery of standard TPP was found to be dependent on the method of elution of TPP from Kiesel Gel DO (Table I-B). Fifteen samples of standard

TABLE I

THE RECOVERY OF TPP UNDER VARIOUS CONDITIONS

(See text for description of sample treatments A, B, C, D.)

| No. of samples        | Absorbancies $\pm$ S.D. ( $\mu\text{g}$ ) |  | % Recovery |
|-----------------------|---|--|------------|
|                       | 515 $m\mu$                                | 481 $m\mu$                                 |            |
| A 18 (std TPP)        | 0.240 $\pm$ 0.013 (23.7 $\mu\text{g}$ )   |  |            |
| B 17 (std)            | 0.237 $\pm$ 0.002 (23.4 $\mu\text{g}$ )   |  |            |
| B 15 (batch elution)  | 0.213 $\pm$ 0.014 (21.0 $\mu\text{g}$ )   |  | 90 %       |
| B 12 (std)            | 0.275 $\pm$ 0.005 (27.1 $\mu\text{g}$ )   |  |            |
| B 14 (column elution) | 0.262 $\pm$ 0.006 (25.8 $\mu\text{g}$ )   |  | 95 %       |
| B 15 (std)            |   | 0.335 $\pm$ 0.012                          |            |
| B 14 (column elution) |   | 0.319 $\pm$ 0.005<br>(0.14 $\mu\text{g}$ ) | 95 %       |
| C 5 (std)             |   | 1.012                                      |            |
| C 5 (irrad)           |   | 0.281                                      |            |
|                       |   | 1.293                                      |            |
| C 5 (mixture A)       |   | 1.203                                      | 93.5       |
| C 5 (std)             |   | 0.925                                      |            |
| C 5 (irrad)           |   | 0.303                                      |            |
|                       |   | 1.228                                      |            |
| C 5 (mixture B)       |   | 1.171                                      | 95.5       |
| C 5 (std)             |   | 1.006                                      |            |
| C 5 (irrad)           |   | 0.298                                      |            |
|                       |   | 1.304                                      |            |
| C 5 (mixture C)       |   | 1.227                                      | 94.5       |
| D 15 (o t mc)         |   | 0.303                                      |            |
| D 5 (+ 20 min)        |   | 0.301                                      |            |
| D 5 (+ 3.5 h)         |   | 0.307                                      |            |
| D 5 (+ 20 h)          |   | 0.300                                      |            |

TPP spotted on TLC plates were scraped off the plates and placed in 3 ml benzene, shaken and centrifuged. The supernatant produced a spectrum with a band at 515  $\mu$  of 0.213  $\pm$  0.014 units of absorbancy. This value was compared with the absorbancy produced by a 25  $\mu\text{l}$  sample dissolved directly in 3 ml of benzene and producing a band of 0.237  $\pm$  0.002 units of absorbancy. A yield of 90 % was obtained using this batch method of elution.

However, if elution was done by microcolumn elution, as described in the experimental procedure, the yield was 95 %. The absorbancies obtained on 14 chromatographed samples was 0.262  $\pm$  0.006 and by direct dissolution of the same amount in benzene, the absorbancy was 0.275  $\pm$  0.005 for 12 samples. The total amount of TPP applied in all cases was 27.1  $\mu\text{g}$ . Lowering the total amount of TPP to 0.14  $\mu\text{g}$  still yields 95 % recovery. Fourteen chromatographed samples produced the Soret Band with absorbancy of 0.319  $\pm$  0.005, as compared with an 0.355  $\pm$  0.012 absorbancy obtained on samples dissolved directly in 3 ml of benzene.

The recovery was further checked by mixing the standard TPP with irradiated mixtures of benzaldehyde and pyrrole and then chromatographing, separately, each component and the mixtures. The average absorbancies, at 418  $m\mu$ , of these different

standards and mixtures was obtained on three different days and are presented in Table I-C.

The effect of the length of air drying of the developed TLC plates was checked by chromatographing 15 plates containing the same amount of standard TPP solution. The material eluted immediately after development of the chromatograms yielded an average absorbancy of 0.303 at the Soret band (418 m $\mu$ ). Five plates eluted after 20 min of drying produced a band at 418 m $\mu$  with an average absorbancy of 0.301; five plates eluted after 3<sup>1</sup>/<sub>2</sub> h produced an average absorbancy of 0.307 and five plates eluted after 20 h produced an average absorbancy of 0.300 units. It has been concluded therefore, that the time of drying after development has no measurable effect on the recovery of TPP (Table I-D).

The examination of other *i.e.* lower portions of TLC plates did not reveal any Soret band producing compounds. Therefore, it might safely be assumed that the separation of TPP is quantitative.

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#### SUMMARY

A method for the isolation and quantitative recovery of  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -tetraphenylporphine using thin-layer silica gel chromatography is presented. Possible sources of error in the method were tested and found to be negligible.

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