

## COMPARATIVE EVALUATION OF PRECOATED SILICA GEL PLATES FOR PREPARATIVE LAYER CHROMATOGRAPHY

*A. N. Campbell and J. Sherma*

Department of Chemistry, Lafayette College, Easton, PA 18042, USA

### SUMMARY

Five commercial preparative layer chromatography (PLC) plates precoated with silica gel layers 1 mm thick were compared on the basis of theoretical plate number ( $N$ ) and resolution ( $R$ ) by developing a test dye mixture with the same mobile phase over a constant distance with dye loadings of 1.0–5.0  $\mu\text{g}$  per zone. It was found that the best performance, on the basis of  $N$  and  $R$  data, was obtained from the new Mallinckrodt–Baker layer with 4.5–5.5- $\mu\text{m}$  spherical particles. With one exception the efficiency and resolution of the other layers correlated with their particle size.

### INTRODUCTION

Classical preparative layer chromatography (PLC) involves migration of a mobile phase by capillary action through a 0.5–2 mm layer of adsorbent, usually silica gel, for the purpose of separating compounds in amounts of 10–1000 mg. Nyiredy has stated in a recent book chapter [1] that the silica materials commonly used for PLC have extremely coarse particle sizes (average approximately 25  $\mu\text{m}$ ) with a wide size distribution (5–40  $\mu\text{m}$ ) and that these properties are a general problem with precoated PLC plates. Nyiredy also states [1] that there are currently no commercially available precoated preparative plates with a favorable average particle size and particle size distribution. Despite these statements, four brands of commercial precoated silica gel PLC plates were obtained with smaller average particle sizes and particle-size ranges than the values given by Nyiredy; another matching his specifications was also obtained. A standard dye mixture was used to evaluate the effect of particle size by comparing theoretical plate number ( $N$ ) and resolution ( $R$ ) on these five layers. It is known that resolution and loading are affected by layer thickness,

so all the layers used were 1 mm thick. Mobile phase development was performed consistently to a distance of 10 cm to standardize the effect of migration distance on zone diffusion.

## EXPERIMENTAL

Five commercial 20 cm × 20 cm precoated preparative plates with 1 mm layers were tested:

- Mallinckrodt–Baker (Phillipsburg, NJ, USA) silica gel SI 1000HPF-PA (3C), with concentrating zone at the bottom and 1.5 cm writing layer at the top;
- Whatman (Clifton, NJ, USA) PK5F silica gel 150 Å, catalog no. 4851-840;
- Analtech (Newark, DE, USA) silica gel GF Uniplates, catalog no. 02013;
- Merck (EM Science, Gibbstown, NJ USA, an affiliate of Merck KGaA, Darmstadt, Germany) silica gel PLC plates with concentrating zone at the bottom, catalog no. 1.13792; and
- Macherey–Nagel (Düren, Germany) SIL-G-100 UV<sub>254</sub>, catalog no. 809 063.

The average particle size and particle size ranges of these layers, as provided by a representative of each company, are given in Table I. All the layers were manufactured from irregular silica gel particles except for the spherical particles of the Mallinckrodt–Baker layers.

**Table I**

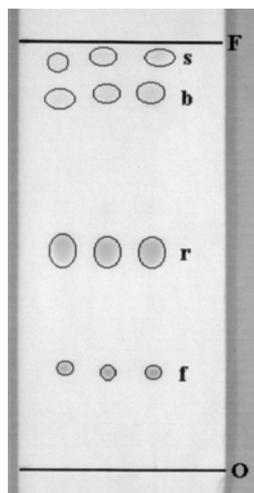
The particle sizes of the PLC layers studied

| Plate brand        | Average particle size (µm) | Particle size range (µm) |
|--------------------|----------------------------|--------------------------|
| Mallinckrodt–Baker | 5                          | 4.5–5.5                  |
| Whatman            | 11                         | >70% 8–16                |
| Analtech           | 15                         | 5–30                     |
| Merck              | 25                         | 5–40                     |
| Macherey–Nagel     | 10–11                      | 5–17                     |

The dye mixture used for evaluation of the layers was Test Dye Mixture I (Analtech catalog no. 30-01) containing 1.0 mg mL<sup>-1</sup> each of Fast Green FCF, Rhodamine B, Bismark Brown Y, and Sudan IV. Initial zones of 1.00, 2.00, 4.00, and 5.00 µL were applied manually, 1.0 cm above the bottom of the plates, by use of a 10-µL Drummond (Broomall, PA, USA) digital microdispenser. Samples were applied in small portions,

with intermediate drying, to keep the initial zone areas as small and uniform as possible. The Mallinckrodt–Baker and Merck plates were evaluated by spotting both on the concentrating zone and on the silica gel layer itself, at opposite ends of the plate. The writing layer was cut off the Mallinckrodt–Baker plates before application of initial zones to the silica gel end opposite the concentrating zone.

Plates were developed with ethyl acetate–methanol–water, 80:20:20, as mobile phase in a Camag (Wilmington, NC, USA) twin-trough chamber containing an Analtech saturation pad. The tank was left to equilibrate for 15 min before inserting the spotted plate. An example of the separation of the dye mixture obtained is shown in Fig. 1. The two lowest zones (blue,



**Fig. 1**

Chromatogram obtained from the four-component dye mixture at 2.0  $\mu\text{g}$  loading separated on a Mallinckrodt–Baker PLC plate by development with ethyl acetate–methanol–water, 80:20:2, as mobile phase. s, Sudan IV (violet–pink); b, Bismark Brown y (yellow); r, Rhodamine B (red), f, Fast Green FCF (blue); F, solvent front; O, origin. The layer was photographed under white light with a Camag VideoStore image-documentation system and the zones were outlined to be easily visible.

$R_F$  0.16, and red,  $R_F$  0.40) were measured on the layer with a millimeter rule to obtain data for calculation of efficiency (theoretical plate number,  $N$ ) and resolution ( $R$ ) data. Each plate was spotted in triplicate at each loading, and individual measurements were averaged. The zones on each track of each layer were scanned at 490 nm by means of the visible mode of a Camag TLC Scanner II densitometer controlled with CATS-3 software.

Densitometer settings included slit length 4 and slit width 4, and a scanning rate of  $4 \text{ mm s}^{-1}$ . The resulting peaks representing the dye zones were measured with a rule in a manner analogous with direct measurements of the zones.

Equations (1) and (2), specified by Kowalska et al. [2], were used to calculate  $N$  and  $R$ , respectively, from direct measurements of dye zones on the layer:

$$N = 16 \times l \times z/w^2 \quad (1)$$

$$R = z_r - z_b/0.5(w_r + w_b) \quad (2)$$

where  $l$  is the migration distance from the origin to the mobile phase front,  $z$  is the migration distance from the origin to the center of the solute zone,  $w$  is the chromatographic zone width in the direction of mobile phase migration, and  $r$  and  $b$  denote the red and blue zones, respectively.

## RESULTS AND DISCUSSION

Table II shows calculated average  $N$  and  $R$  values for three trials of the red and blue dye zones on the five layers studied with loadings of 1.0–5.0  $\mu\text{g}$  applied directly to the silica gel layer. It is apparent that the Mallinckrodt–Baker layer, for which the mean particle size and particle-size range were smallest and which was composed of spherical particles, consistently afforded better  $N$  and  $R$  values for both dyes than the other four plates. As expected,  $N$  and  $R$  decreased regularly with higher loading for this layer.

For the other four layers theoretical plate number and resolution were significantly lower than for the Mallinckrodt–Baker layer. The  $R$  values for these layers decreased in the order Whatman > Analtech > Merck > Macherey–Nagel for all loadings except 1.0  $\mu\text{g}$ , for which resolution on the Macherey–Nagel layer was higher than on the Merck layer. The decrease in  $R$  correlates with increasing average particle size except for the Macherey–Nagel layers (Table I). The reason for this one discrepancy is not known. The  $N$  values for the dyes do not exactly follow the same order as the  $R$  values for the four layers, probably because of inevitable experimental error in zone measurement. The blue zone was usually quite compact, so variations in width were small and probably reflect measurement errors more than for the red zone. The variations in the width of the red zones were much greater from plate to plate, because of its higher  $R_F$

**Table II**

Average number of theoretical plates ( $N$ ) and resolution ( $R$ ) for dyes applied directly to the silica gel layers

| Plate brand        | Weight ( $\mu\text{g}$ ) | Average $N$ of b <sup>a</sup> | Average $N$ of r <sup>a</sup> | Average $R$ |
|--------------------|--------------------------|-------------------------------|-------------------------------|-------------|
| Mallinckrodt–Baker | 1.00                     | $3.20 \times 10^4$            | $5.42 \times 10^3$            | 11.40       |
|                    | 2.00                     | $1.42 \times 10^4$            | $2.42 \times 10^3$            | 8.18        |
|                    | 4.00                     | $6.36 \times 10^3$            | $1.58 \times 10^3$            | 6.63        |
|                    | 5.00                     | $1.19 \times 10^3$            | $1.23 \times 10^3$            | 5.69        |
| Whatman            | 1.00                     | 892                           | 796                           | 5.42        |
|                    | 2.00                     | 853                           | 555                           | 6.17        |
|                    | 4.00                     | 770                           | 806                           | 5.23        |
|                    | 5.00                     | 296                           | 863                           | 4.68        |
| Analtech           | 1.00                     | 769                           | 375                           | 4.45        |
|                    | 2.00                     | 587                           | 211                           | 3.54        |
|                    | 4.00                     | 214                           | 238                           | 3.72        |
|                    | 5.00                     | 316                           | 163                           | 2.66        |
| Merck              | 1.00                     | 224                           | 350                           | 1.75        |
|                    | 2.00                     | 566                           | 336                           | 3.34        |
|                    | 4.00                     | 197                           | 262                           | 2.26        |
|                    | 5.00                     | 409                           | 153                           | 2.35        |
| Macherey–Nagel     | 1.00                     | 61.2                          | 747                           | 2.12        |
|                    | 2.00                     | 49.0                          | 469                           | 2.31        |
|                    | 4.00                     | 36.2                          | 867                           | 1.35        |
|                    | 5.00                     | 83.7                          | 346                           | 2.22        |

<sup>a</sup> b denotes the blue zone and r the red zone

value and greater diffusion, and  $N$  values for this dye probably represent the relative qualities of the plates better than those for the blue dye. For example, for 5.0  $\mu\text{g}$  loading the difference between the widths of the blue zones on the Merck and Mallinckrodt–Baker layers was only 1.0 mm, whereas the difference between the widths of the red zones was 15.0 mm and, therefore, not as subject to measurement errors.

Data comparable with those in Table II were collected by measuring results from densitometric scanning, instead of the zones directly. For these the maximum of the peak was used as the center of the zone, and the zone width was measured as the width of the peak at the baseline. Values of  $N$  and  $R$  calculated from results from densitometric scanning were lower, because the densitograms exaggerated the extents of the leading and trailing edges of the zones at the baseline compared with measurement of zone widths directly on the layer. Comparable zone-center measure-

ments were obtained by the direct and densitogram procedures. The trends were the same for the two measurement procedures and the Mallinckrodt–Baker plate proved to be consistently the best.

The initial zones were applied in small volume increments with intermediate drying to keep their sizes as small and uniform as possible. The initial zones for the 1.00–5.00- $\mu\text{L}$  volumes ranged from 2.0–4.0 mm, with a mean of 3.1 mm and relative standard deviation (*RSD*) of 19%. Loadings of 1.00–5.00  $\mu\text{g}$  in spots of this size are representative of the higher loadings applied in streaks across the layer width in actual PLC separations.

The Mallinckrodt–Baker and Merck plates contained a concentration zone spotting area, and the dye mixture was spotted at the same 1.0–5.0  $\mu\text{g}$  loadings on the concentration zones of these plates to compare performance with that shown in Table II for application directly to the silica gel layer. The blue and red dye zones could be used for this comparison only at 1.0  $\mu\text{g}$  loading, because the blue dye did not migrate completely out of the concentration zone at higher loadings. By comparing Tables II and III it can be seen that at 1.0  $\mu\text{g}$  loading the performance of the Mallinckrodt–Baker layer was better when application was directly on the layer, whereas the Merck layer gave better results when application was made to the concentration zone. The effect of the concentration zones was further evaluated by measuring *N* and *R* values for the upper yellow ( $R_F$  0.72) and violet–pink ( $R_F$  0.84) zones for 1.0–5.0  $\mu\text{g}$  loadings. Both of these dyes migrated with these  $R_F$  values when spotted on the concentration zone or directly on the silica gel layer at the other end. The data indicated that *N* and *R* values for both layers were similar irrespective of where the initial zones were applied, and the performance of the Mallinckrodt–Baker layer was again much better than that of the Merck layer.

**Table III**

Average number of theoretical plates (*N*) and resolution (*R*) for dyes applied to the concentration zones at 1.0  $\mu\text{g}$  loading

| Plate brand        | Average <i>N</i> of b <sup>a</sup> | Average <i>N</i> of r <sup>a</sup> | Average <i>R</i> |
|--------------------|------------------------------------|------------------------------------|------------------|
| Mallinckrodt–Baker | $3.76 \times 10^4$                 | $1.61 \times 10^3$                 | 7.80             |
| Merck              | 357                                | $1.28 \times 10^3$                 | 2.43             |

<sup>a</sup> b denotes the blue zone and r the red zone

It has been shown in this study that 1-mm PLC layers with small mean particle size are available commercially, and that the new Mallinck-

krodt–Baker layer with 4.5–5.5- $\mu\text{m}$  spherical particles performs better than the other layers tested in terms of  $N$  and  $R$  values for a test dye mixture separated by use of constant TLC conditions. Layers prepared from 5- $\mu\text{m}$  particles are common in modern high-performance thin-layer chromatography (HPTLC) plates, because in these layers separation efficiency is optimum [3]. The only other precoated plates available today with layers prepared from spherical silica gel particles are two HPTLC products manufactured by Merck [4]. LiChrospher HPTLC Si 60 layers F<sub>254s</sub> are prepared by applying 6–8- $\mu\text{m}$  particles with 60-Å pores to glass (0.2 mm layer thickness) and aluminum (0.1 mm). Their advantages are separations approximately 20% faster and more compact zones than for HPTLC plates with irregular particles. LiChrospher HPTLC Si 60 F<sub>254s</sub> Raman layers, which are made for in-situ Raman spectrometry, are coated with 0.1 mm layers of 3–5- $\mu\text{m}$  particles on an aluminum support. The spherical silica gel results in signal intensity ten times higher than that from similar layers prepared from irregular silica gel particles. Given the advantages of analytical HPTLC layers prepared from small-diameter, spherical silica gel particles, it is not surprising that the Mallinckrodt–Baker PLC plates manufactured from this material proved to be best in this comparative study.

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