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C. Estimation of Preparative Sample Load

The quantity of sample which can be loaded onto a preparative column is a function of the separation initially achieved on the TLC plate. Changing the TLC conditions to increase the separation will significantly increase the amount of sample that can be loaded onto the preparative LC. Using the initial TLC conditions, a preparative separation can be performed following the guidelines listed in Table 4.

Table 4. Guidelines for Estimating Load

(R_f) 1st Component	**(R_f) 2nd Component**	**ΔR_f**	**Load g/Component**	**Typ. Sample grams**
0.30	0.15	0.15	6.0	10-20
0.30	0.20	0.10	3.5	5-10
0.30	0.23	0.07	2.0	3-5
0.30	0.25	0.05	0.8	1-2

Example: Figure 4a illustrates a TLC separation with the first component located at an $R_f = 0.3$, the second component located at an $R_f = 0.15$. The corresponding LC separation is illustrated in Figure 4b. With this degree of separation, up to a 20g sample can be loaded on a single PrepPAK® 500 Cartridge.

Figure 4 Comparison of TLC and LC Separation for a $\Delta R_f = 0.15$

Fig. 4a

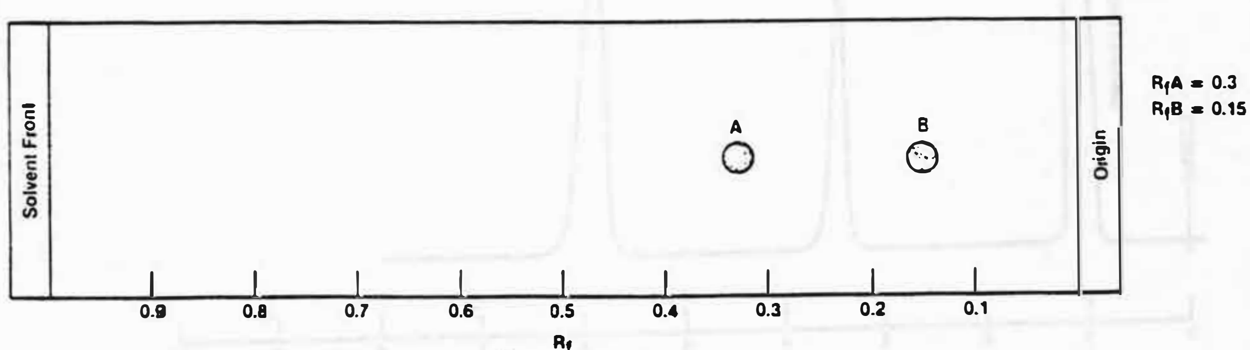
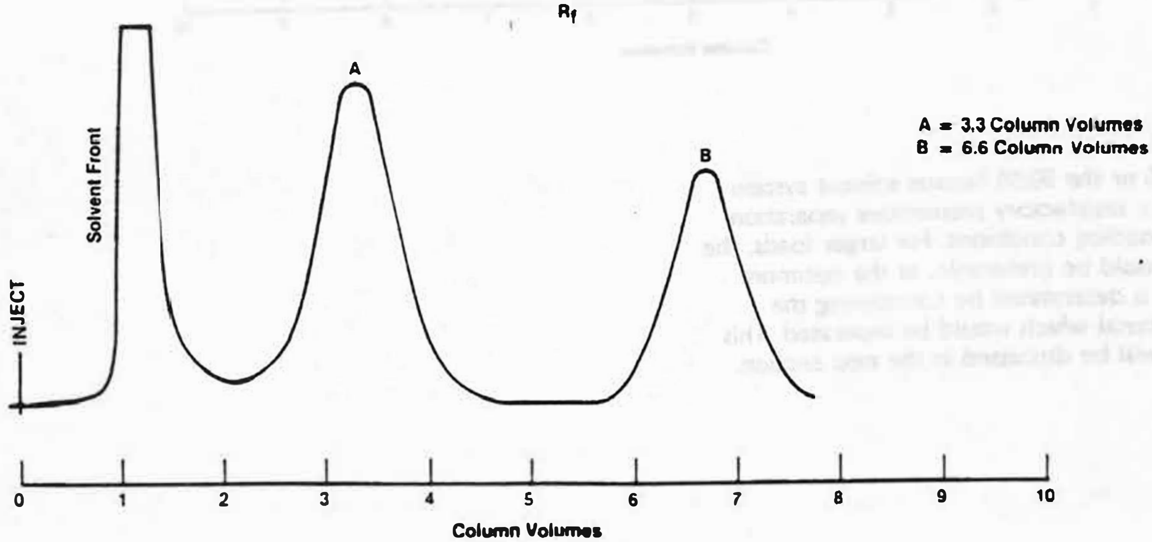


Fig. 4b



When the TLC separation does not provide as much separation (Table 4), and the second component has an $R_f = 0.2$ (Fig. 5a), a sample of 6g or less can be separated (Fig. 5b).

Figure 5 Comparison of TLC and LC Separation for a $\Delta R_f = 0.1$

Fig. 5a

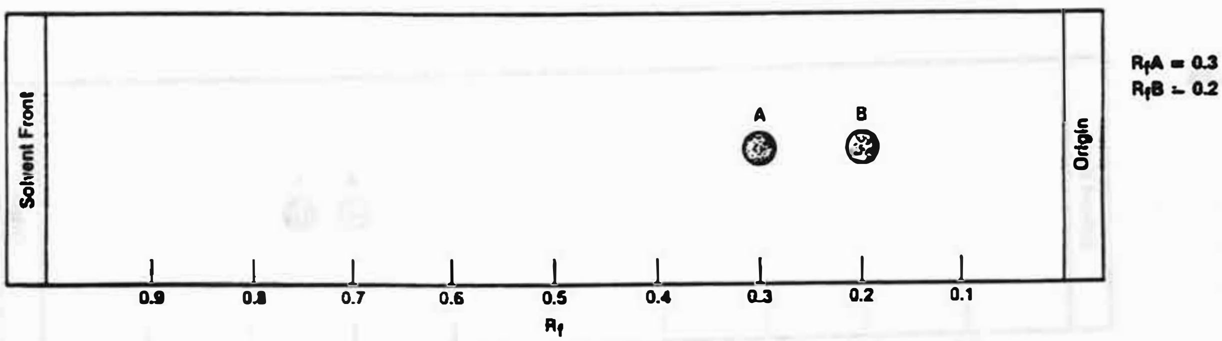
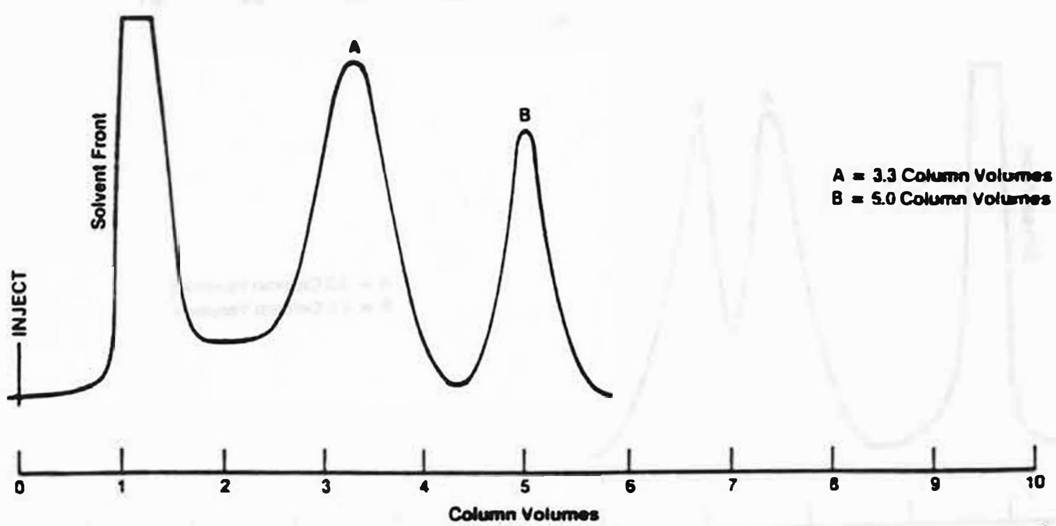


Fig. 5b



If the TLC separation is minimal, with the two components not fully resolved, the preparative LC can separate 1-2g (Fig. 6a, b). If higher sample loading is desired, the separation in a preparative system can be optimized through recycle chromatography to provide a two- or three-fold increase in sample load per separation, while minimizing the solvent consumption.

Figure 6 Comparison of TLC and LC Separation for a $\Delta R_f = 0.05$

Fig. 6a

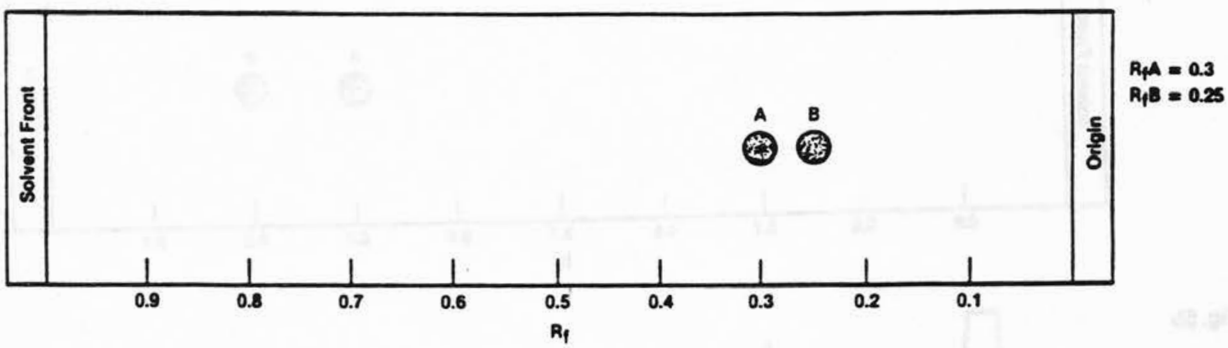
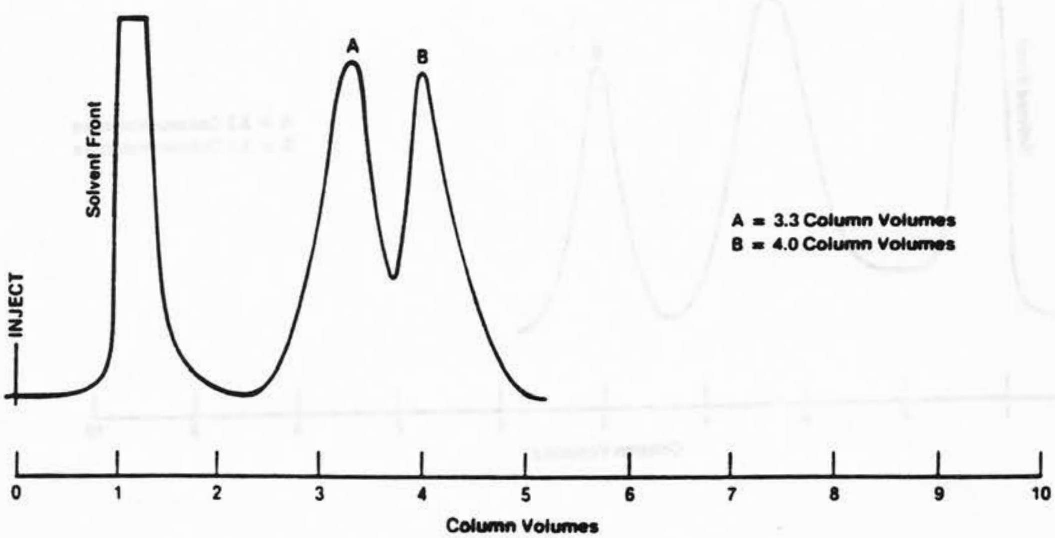


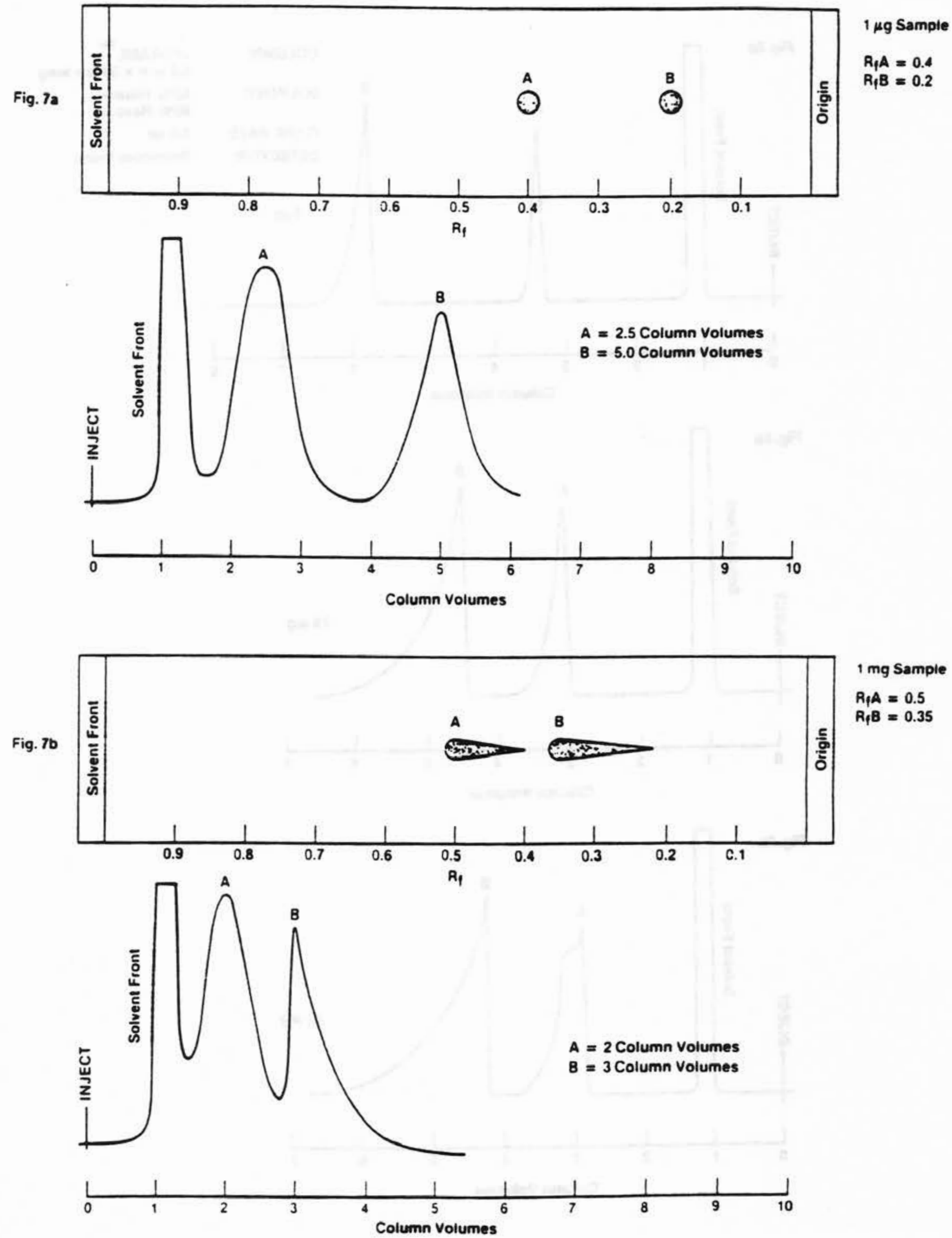
Fig. 6b



D. Effect of Increased Sample Load

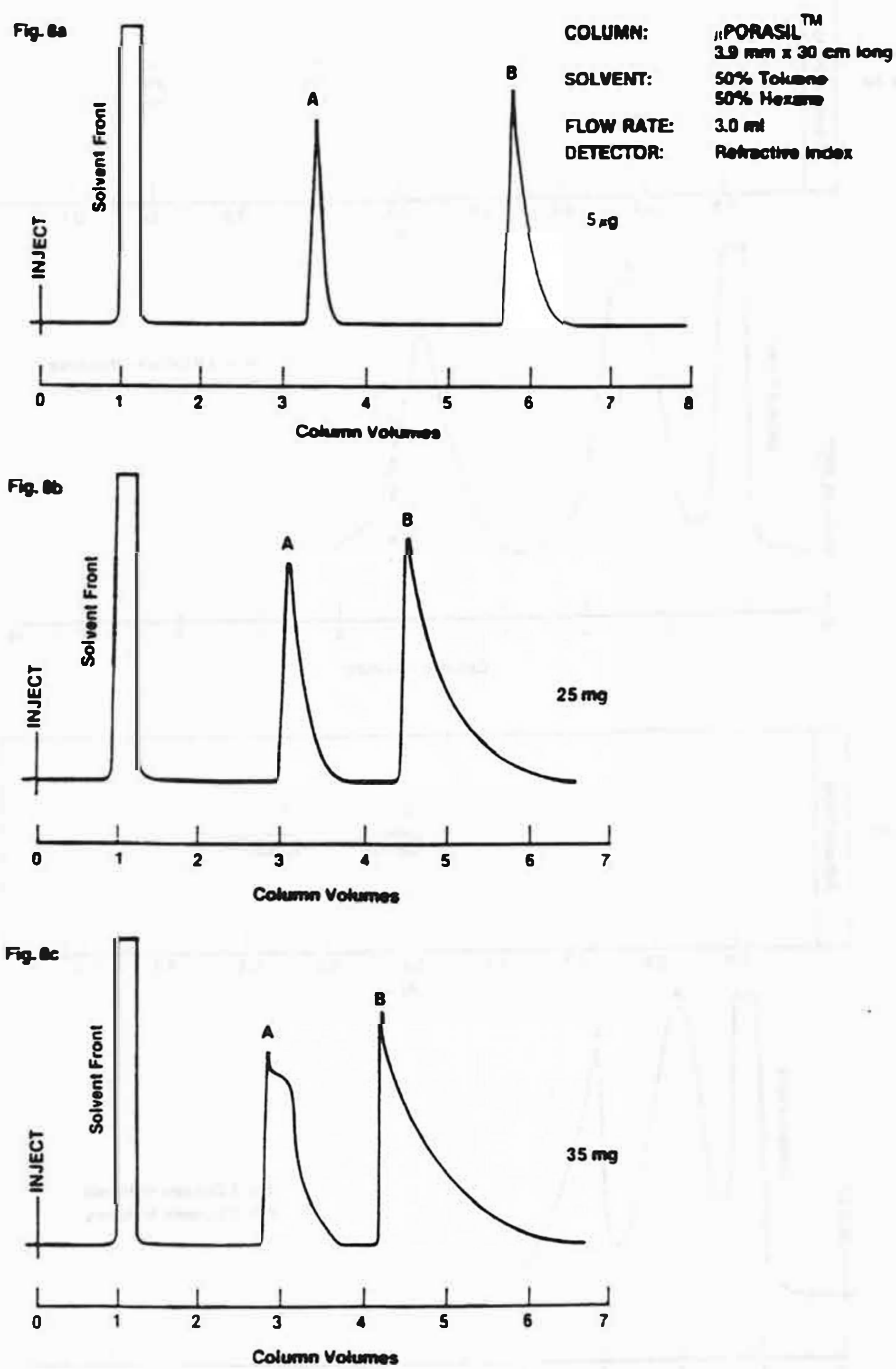
When the sample size is increased from one microgram to one milligram, the major components travel further up the TLC plate and the spots are skewed. This occurs because the silica sites on the stationary phase are saturated with sample, resulting in an increase in the R_f value and a corresponding decrease in the column retention volume on the LC (Fig. 7).

Figure 7 Comparison of TLC and LC Separation for a 1 µg Sample and 1 mg Sample



Example: To illustrate the effect sample load has on the C.V., the *cis-trans* isomer mixture, previously separated by TLC (Fig. 3), is now separated on the analytical LC. In Fig. 8a, the first chromatogram shows a few micrograms of the sample separated. By increasing the sample load to 25 mg, the silica sites start to saturate and the peaks elute faster (Fig. 8b). If the sample size is increased further to 35 mg, the two compounds will elute even sooner (Fig. 8c). The peak centers are distorted towards the front, and a corresponding skewed or tailing peak is formed. Although the column volumes have changed with the increased sample size, the HPLC separation is still sufficient to allow a 7g preparative separation.

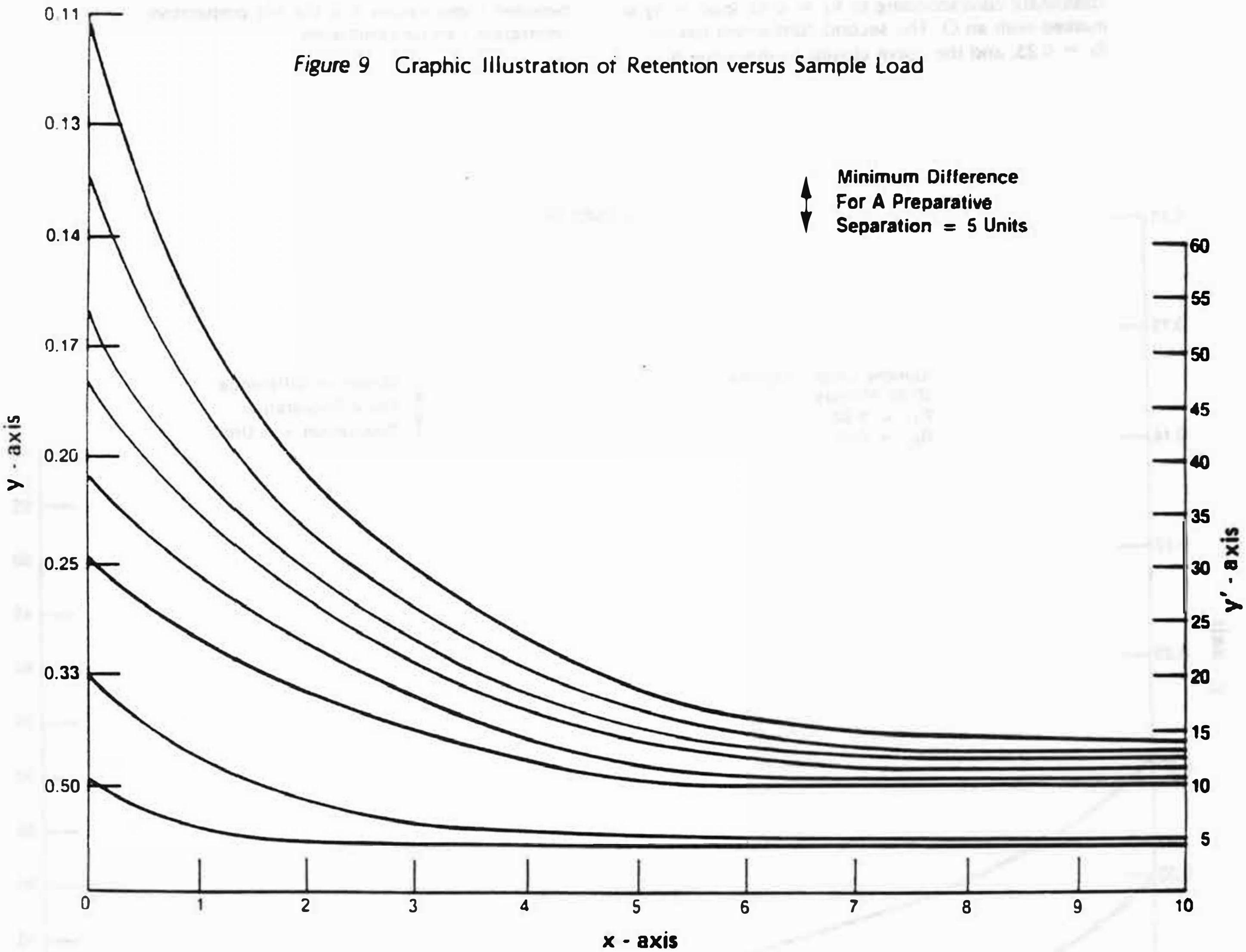
Figure 8 Effect of Sample Load on Retention Volume



Generally, the R_f should be adjusted toward 0.1-0.15 for increased sample loads and more difficult separations ($\Delta R_f < 0.07$).

As previously mentioned, when the sample weight is increased the components travel further up the TLC plate, or in a column the components elute faster. When only TLC data is available, prior to a preparative separation, the sample load can be estimated utilizing Table 4 or Fig. 9.

Figure 9 Graphic Illustration of Retention versus Sample Load



The curves in Fig. 9 were generated with model compounds and graphically illustrate the effect load has on the retention of a compound. The following four steps may be used to estimate the preparative sample load.

1. Locate the R_f for the first component on the left-hand ordinate (y-axis). Follow the curve nearest this point to the right until it intersects the approximate weight for that component on the x-axis. Mark these coordinates on the graph.

2. Repeat step one for the second component and mark the second set of coordinates on the graph.

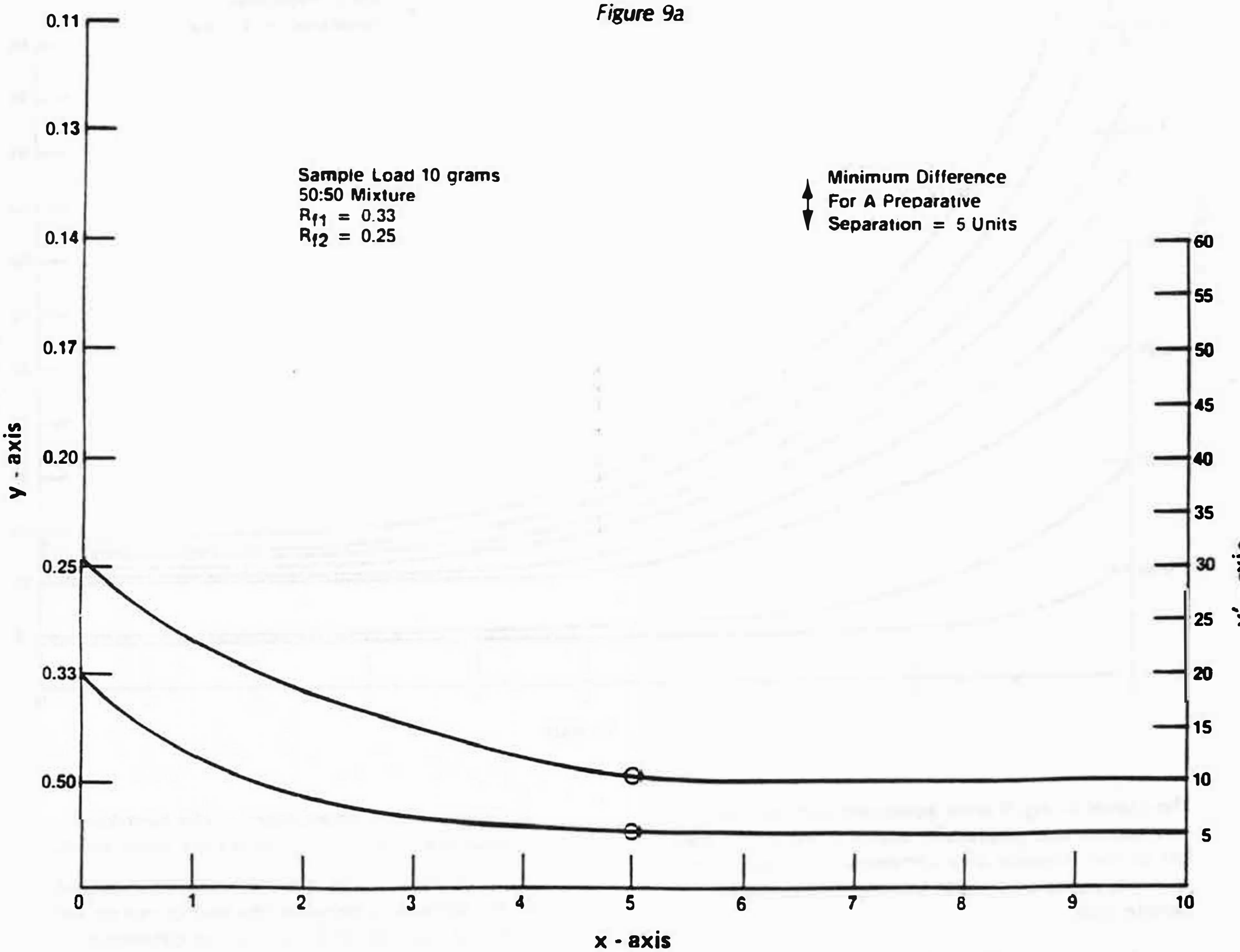
3. Transpose the coordinates to the right-hand ordinate (y′-axis) for each sample component.

4. To assure a successful preparative separation, the difference between the two points on the y′-axis must be at least 5. If the difference between the two components on the y′-axis is less than 5, the total sample load must be decreased. If the distance is greater than 5, this sample can be chromatographed or the sample load can be increased further for optimal results.

The following three examples illustrate the use of Fig. 9 to estimate sample load for a 10g sample containing two compounds with R_f's = 0.33 and 0.25.

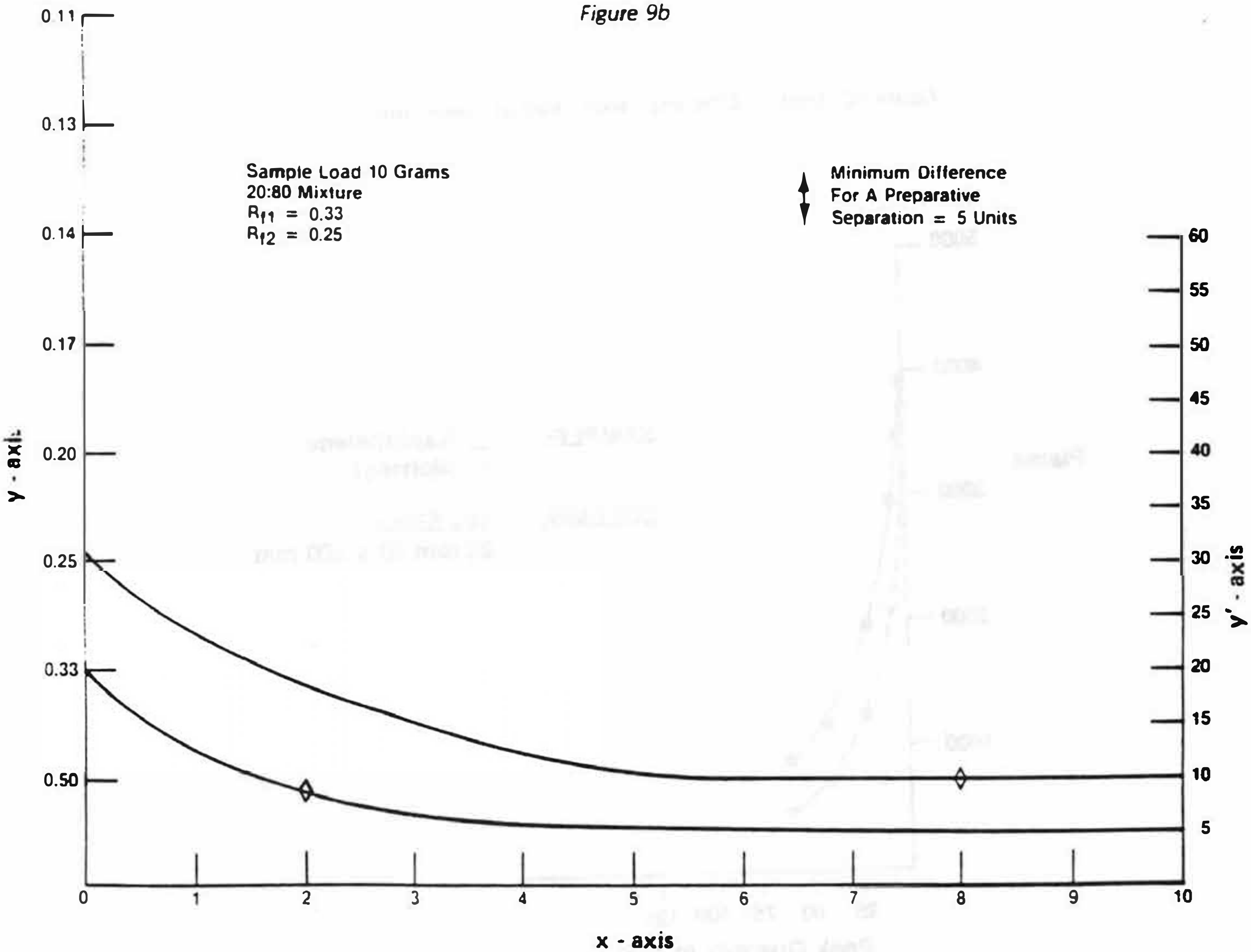
Example A: The 10g mixture is comprised of a 50:50 mixture. In Fig. 9a the R_f of the first component is 0.33. The curve closest to this value is followed until the 5g sample load is reached on the x-axis. The coordinate corresponding to R_f = 0.33, load = 5g is marked with an O. The second component has an R_f = 0.25, and the curve closest to this value is followed until the 5g sample load is reached on the x-axis. The coordinate corresponding to this value is marked with an O. Both these points are transposed to the y′-axis at 11 and 6. Since the difference between these values is 5, the 10g preparative separation can be completed.

Figure 9a



Example B: The 10g mixture is comprised of a 20:80 mixture. In Fig. 9b the R_f of the first component is 0.33. The curve closest to this value is followed until the 2g sample load is reached on the x-axis. The coordinate corresponding to R_f = 0.33, load = 2g is marked with a ◊. The second component has an R_f = 0.25, and the curve closest to this value is followed until the 8g sample load is reached on the x-axis. The coordinate corresponding to this value is marked with a ◊. These points are transposed to the y'-axis at 10 and 8. The difference between these values is less than 5, so the sample size must be decreased to 5g before doing the preparative separation.

Figure 9b



Example C: There is dramatic loss of theoretical plates, N, when loading small particle silica columns with compound. From this example, a 20 mm x 30 cm column packed with 10 micron packing was very efficient at low loads, but at 50 mg of load efficiency became less than 1000 theoretical plates. A column of this size is designed for operation in the 100 to 500 mg range based on a 100 to 1 silica to sample loading ratio. Substitution of 37 to 55 micron packing material can give the equivalent performance in the 100 to 500 mg loading range without the back pressure, flow limitations or cost incurred by use of 10 micron material.

Figure 9C Loss of Efficiency with Load of Compound