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IV. Preparative LC Solvent Systems

A. Ethyl Acetate or Less Polar Solvents

Ethyl acetate provides good solubility for many samples, while having a moderate affinity for the silica gel. It is widely used to remove polar contaminants from the silica and may extend the lifetime of the cartridge. The column can be rapidly equilibrated with the next mobile phase, and ethyl acetate is also easily distilled from collected fractions.

When the TLC solvent system contains ethyl acetate, the mobile phase is adjusted to provide an $R_f = 0.5$. The R_f for the compound can be quickly readjusted to 0.3-0.25 by utilizing a solvent system containing only 1/3 the ethyl acetate.

Example: If the initial solvent contained 60% EtOAc/40% MeCl₂, the optimized preparative solvent would be 20% EtOAc/80% MeCl₂. This one solvent system can be rapidly checked by TLC before performing the preparative separation.

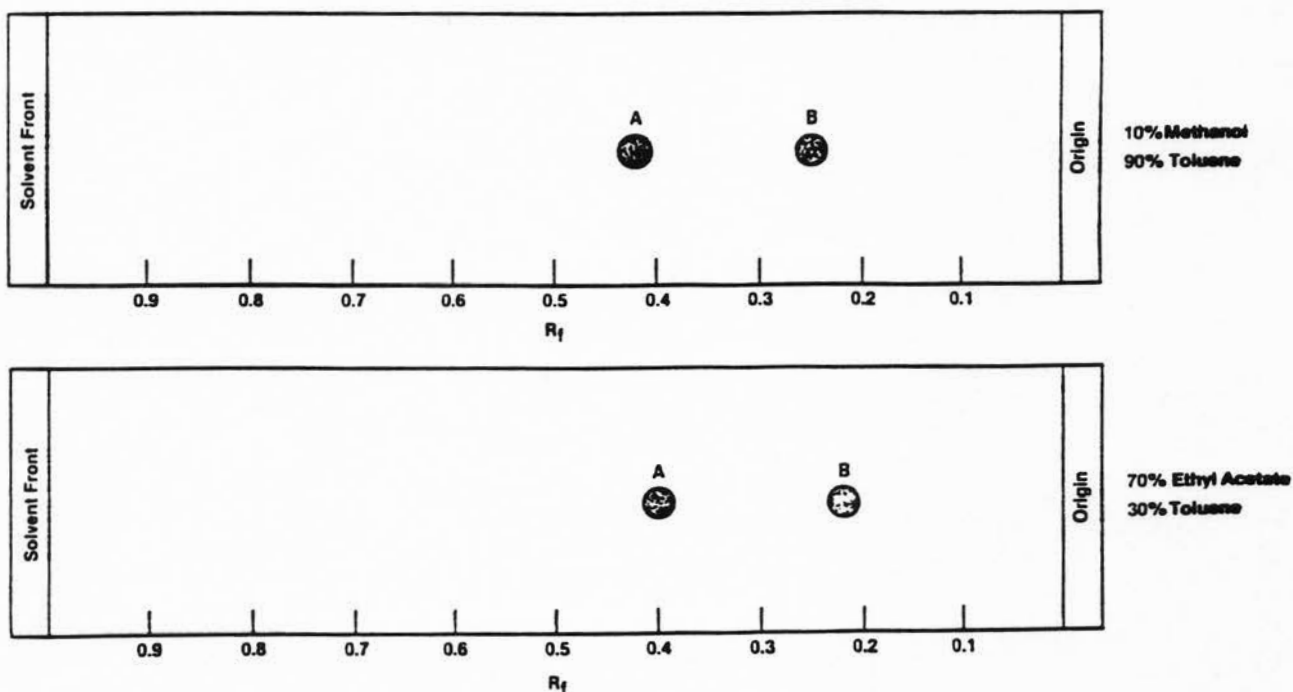
Table 5 provides a convenient way to alter solvent composition to utilize ethyl acetate while maintaining the solvent polarity. CAUTION: While finding a more appropriate solvent for preparative purposes, a change in either solvent may alter the resolution.

Table 5 Relationship of Ethyl Acetate to Other Polar Solvents

Ethyl Acetate	1 to 1
Methylethyl Ketone	1.7 to 1
Acetone	2.7 to 1
n-Butanol	3-4 to 1
n-Propanol	4-5 to 1
Ethanol	5-6 to 1
Methanol	7 to 1

Example: Table 5 explains that the amount of ethyl acetate in toluene required to produce an equivalent polarity is 7 times the amount of methanol required for the same results (Fig. 10).

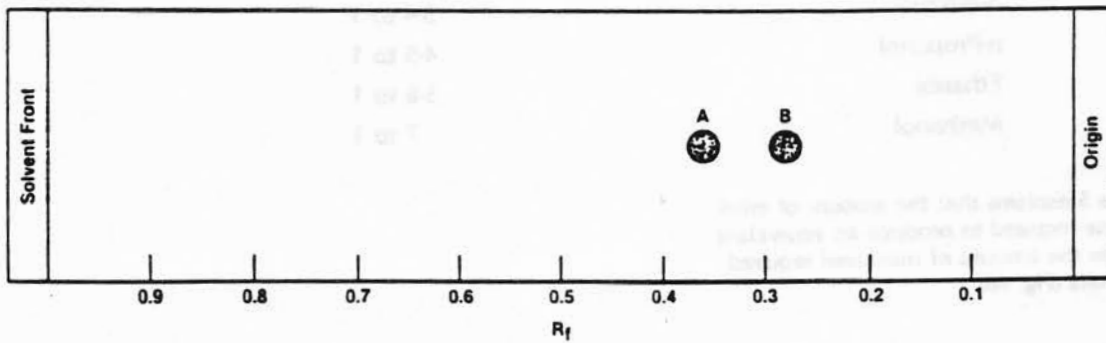
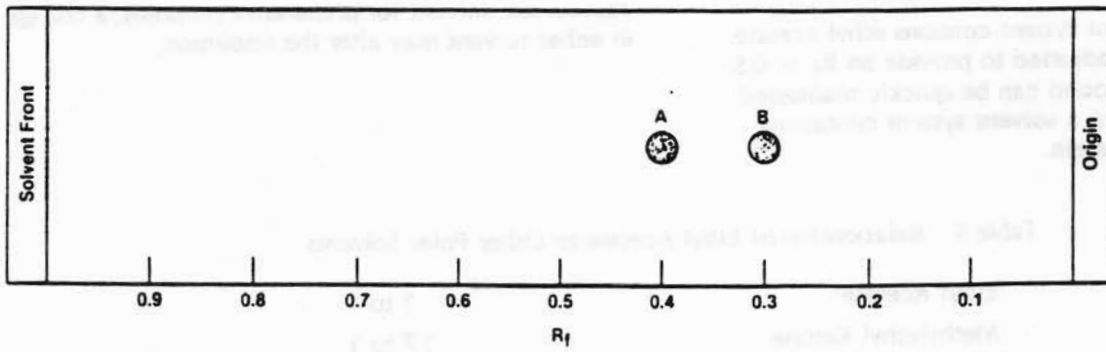
Figure 10 Conversion of Solvent Systems Based on Relative Polarity



The original TLC separation is 10% methanol 90% toluene.

The same solvent polarity is achieved with 70% ethylacetate 30% toluene.

Figure 10 (continued)



The original TLC separation is 50% ethyl acetate, 50% hexane.

The same solvent polarity is achieved with 20% acetone, 80% hexane.

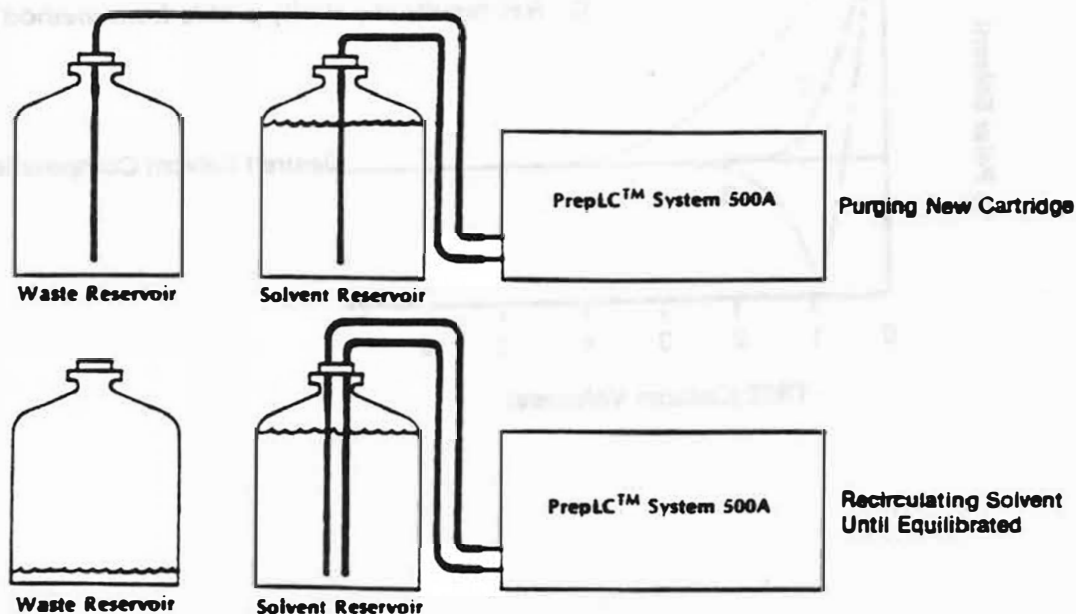


B. Equilibration of the Preparative LC Columns

When the preparative solvent mixture contains solvents no more polar than ethyl acetate (Table 1) and contains greater than 5% of the polar solvent, the preparative columns are equilibrated rapidly. Two column volumes of the preparative solvent are passed through the cartridge and discarded (Fig. 11). The

waste line is then diverted to the solvent reservoir and the solvent is recirculated through the column. Continuous stirring of the solvent mixture ensures rapid and continuous equilibration between the solvent and column. Generally, the solvent is equilibrated when the refractive index detector is stable on the 20X sensitivity setting.

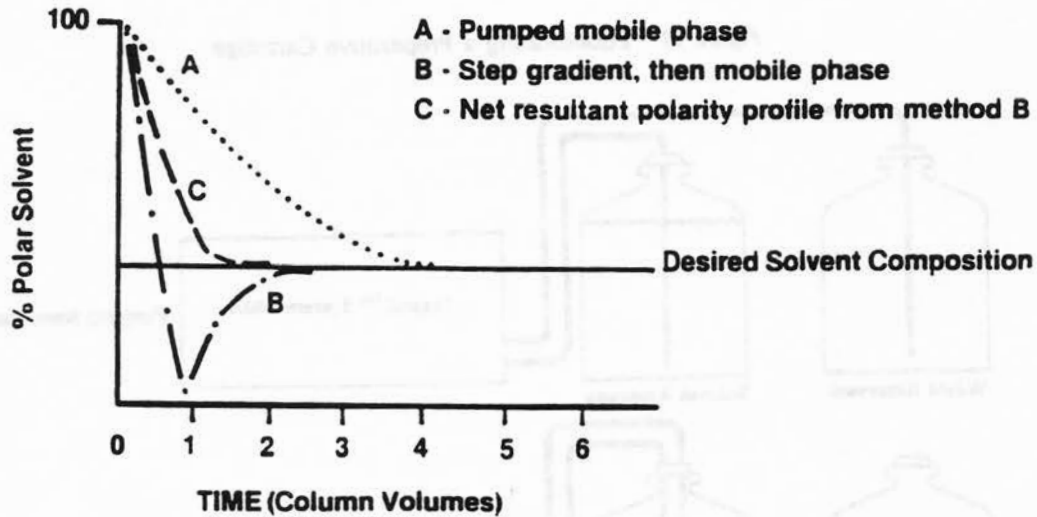
Figure 11 Equilibrating a Preparative Cartridge



Silica gel has a greater affinity for the polar solvent and the polar solvent is preferentially adsorbed on the silica. When less than 5% of the polar solvent is present, a new silica cartridge will adsorb significant quantities, changing the solvent polarity. This can be prevented by purging the column initially with two column volumes of solvent containing 10-15% of the polar solvent. After purging, the preparative LC solvent is introduced, and the column is equilibrated as listed above.

After equilibrating the column, the preparative LC solvent is rapidly evaluated by developing a TLC plate with this solvent. If the compounds elute within the appropriate R_f range, the preparative separation is performed. If the compounds do not elute within the appropriate R_f range, changes in solvent polarity can be made to adjust the R_f and the columns reequilibrated with the new solvent.

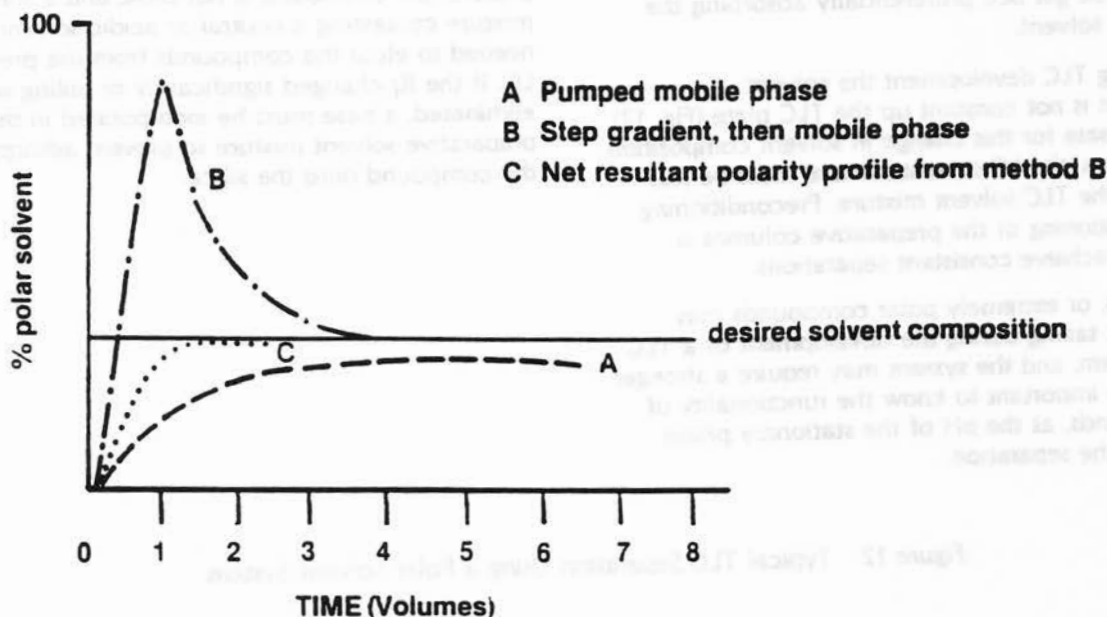
Figure 11B Equilibration of Columns less Polar than the Desired Solvent Composition



For silica columns which have been flushed and stored in a polar solvent and are thus deactivated, or for bonded phase columns, the equilibration process consists of sweeping out the deactivating agents and wetting the surface with the solvent of choice. Other than pumping the desired mobile phase to accomplish this task (curve A), a more economical approach is to first pump one column volume of a solvent less polar than the desired solvent composition and then switch to the desired mobile phase and

pump two column volumes of it to rapidly bring the column to its point of equilibration (curve B). When the refractometer becomes asymptotic to a base line (curve C), discontinue sending solvent to waste and begin recirculating solvent to the reservoir. After several minutes of equilibration remove some solvent to test by the analytical procedure to determine that the solvent composition in the reservoir is indeed the desired solvent composition.

Figure 11A Equilibration of Columns More Polar than the Desired Solvent Composition



Because the column packing material is more polar than the solvent composition, it will tend to adsorb the polar constituents from the solvent, thus lowering solvent's overall polarity during the equilibration process (curve A). To compensate for this phenomenon, one must first saturate the packing material with the polar constituents of the solvent using one column volume of a solvent more polar than the desired solvent composition. After this switch to the mobile

phase of choice, flush two column volumes of it through the column and into waste (curve B). When the refractometer becomes asymptotic to a base line (curve C), discontinue wasting the solvent and begin recirculating solvent into the reservoir. After several minutes of equilibration, remove some solvent to test by analytical conditions to determine that the solvent composition in the reservoir is the desired solvent composition.

C. Solvent Systems More Polar Than Ethyl Acetate

When the mobile phase contains solvents equal to or less polar than ethyl acetate, correlation from TLC to preparative LC is good. As the affinity of the solvent for the packing material increases, correlation from TLC to preparative LC becomes less precise due to the TLC silica gel bed preferentially absorbing the more-polar solvent.

Thus, during TLC development the solvent composition is not constant up the TLC plate (Fig. 12). To compensate for this change in solvent composition on TLC plates, the LC solvent mixture must be less polar than the TLC solvent mixture. Preconditioning and reconditioning of the preparative columns is required to achieve consistent separations.

Acids, bases, or extremely polar compounds may exhibit peak tailing during the development of a TLC solvent system, and the system may require a stronger solvent. It is important to know the functionality of the compounds, as the pH of the stationary phase may affect the separation.

To test the effect pH has on the separation, a small container of ammonium hydroxide can be placed within the TLC chamber. The ammonia vapors alter the pH on the silica surface without changing the TLC solvent composition. If the component's R_f did not change significantly with the ammonium hydroxide present, the compound is not basic and a solvent mixture containing a neutral or acidic solvent will be needed to elute the compounds from the preparative LC. If the R_f changed significantly or tailing was eliminated, a base must be incorporated in the preparative solvent mixture to prevent adsorption of the compound onto the silica.

Figure 12 Typical TLC Separation Using a Polar Solvent System

