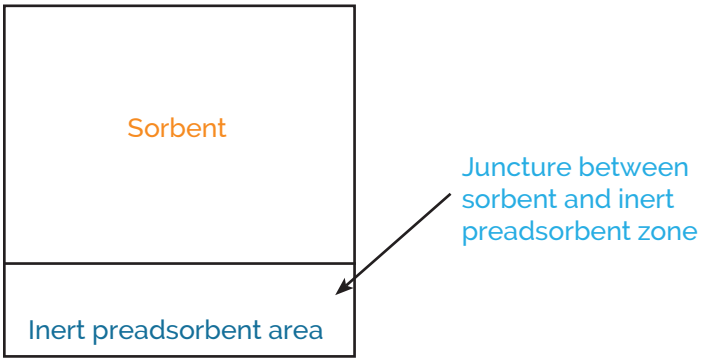
Using Predevelopment to Improve Thin Layer Chromatographic Separations

It is considered good practice to apply samples to a TLC layer in the form of a band rather than a spot. Bands tend to provide better resolution, especially between analytes with close R_f values. Methods of applying samples as bands often require special automated application devices or, at least, skillful manipulation of a manual application device. However, there are several ways to apply samples as bands without such difficulties.

The easiest way to accomplish this is to use a plate with a preadsorbent zone. These plates feature a 2cm wide strip of inert sorbent at the bottom of the plate as illustrated below. Samples applied here will migrate in the mobile phase without separation. When they migrate to the juncture of the preadsorbent and the silica gel, they are forced into bands. Thus, a sample may be applied to the layer with little care, even with respect to applying them in a horizontal line, and they will still form bands.



This can also be accomplished on layers without preadsorbent zones by using a predevelopment technique and appropriate solvents. First apply the samples across the origin of the TLC plate. Dry the area, then place it in a developing tank containing a mobile phase (pre-developer) which is known to cause the sample to migrate with the solvent front. When using normal phase sorbents, this usually can be a solvent of higher polarity than the mobile phase used for the development. For reversed phase sorbents, it will usually be a solvent of lower polarity than the mobile phase used for the development.

Allow the pre-developer to wick up the plate until the samples have been forced into bands at the origin. Do not develop above the origin.. Remove the plate from chamber and dry. Then, develop in the desired mobile phase.

Predevelopment is probably the most overlooked technique in TLC. However it can be used to overcome a variety of difficulties. A few are listed below:

1. When the applied zone is too diffuse to permit proper chromatography. This situation exists often when sample solutions are too dilute.
2. Predevelopment provides a means of extracting the sample directly on the layer. Preadsorbent layers are best used here. The extracting solvent is allowed to develop to the origin. This can be repeated several times to completely extract the sample.
3. Predevelopment can be used to separate compound classes by judicious use of the mobile phases. This is a form of multidimensional development. Polar materials can be separated from the material. Then, two separate developments can be used to separate the individual components in each class.
4. Predevelopment should be requisite in two dimensional development. After the separation in the first direction it is desirable to predevelop the separations into the new origin for the second development. The reason for this is that separated zones are generally too diffuse in the first dimension for satisfactory resolution in the second dimension.
5. The technique has been used to extract biological samples, soil, crude petroleum, environmental contaminated samples etc. on the layer.
6. It is useful to remove reaction products from reactions carried out on the layer. This will deposit the product(s) as a band on the origin.

Several procedures should be followed when using the pre-development technique:

7. The samples should not be so concentrated that the reagent or solvent cannot diffuse through the layer and extract the sample.
8. Between each step the layer (or sorbent) must be dry. Solvent within the matrix will effect the concentration or nature of the predeveloping solvent. Or it can interfere with any reaction to be performed.
9. The predeveloper generally should be more polar than the developing mobile phase to be used. This is required to move the sample to a band at the origin.
10. For HPTLC no more than 100ng total sample should be used. For conventional layers, 1-5µg is the limit. These prerequisites depend on the sensitivity of detection. As a general rule, apply as little sample as is commensurate with detecting the desired analyte.