be generally higher on the plate. Hence, some reoptimization of the developing solvent may be necessary to reduce the migration and possibly restore some of the lost resolution.

A frequently asked question is, how much can be loaded on these plates? The scale-up mentioned above is true, but the absolute amounts have to be experimentally determined. This is done by increasing the amounts spotted (or streaked) on a few preparative plates. Each mixture (amounts of each compound), the resolution (spots well separated or near one another), and the solvent system (which has to successfully dissolve the increased amounts of sample and still resolve the components) all play a role in the final loadability of any preparative plate.

Some preparative TLC applications include azo dyes (275), coumarins (276), plant components (277,278), and triterpenoids (279).

VII. BINDERS IN PREPARED TLC PLATES

To form a rugged TLC surface—one that can be spotted, developed, and visualized without damage—a binder has to be incorporated into the slurry formulation when the plates are being made. When chromatographers began using TLC plates and had to make their own, the traditional binder used was gypsum (G coding) or calcium sulfate hemihydrate (the very familiar plaster of Paris). It was used in about a 10–15% by weight proportion in the silica gel. After mixing and pouring or casting onto a glass plate, the slurry goes from a shiny wet look to a flat finish. This is the first stage of the drying and setting up of the calcium sulfate to form a dihydrate. Further air drying completes the plate manufacture. Note that the plate appears dry at this stage but still contains a great deal of water associated with the silanols. Heat activation is still necessary to remove the absorbed water.

Although the gypsum helps keep the silica gel on the glass plate, it is a very fragile binder, and such layers were called "soft" layers. Care in all the steps of TLC had to be taken so as not to disturb the layer and cause poor chromatography or loss of some of the components. Often, after visualization, the plates were sprayed with a polymeric fixative (such as a polyvinyl alcohol). Other binders such as silicate solutions and starch have also been used, but these were never as popular as the gypsum binder.

Aware that "soft" layer TLC plates were difficult to ship, various TLC plate manufacturers began experimenting with alternative binders. Most settled on various water-soluble polymeric binders to replace gypsum. The result was a much more durable layer that could be stacked for easy shipment and written on with a soft lead pencil to keep track of samples and TLC conditions. These are often referred to as "hard" layer plates.

Although the binders used are proprietary, they are related to polyvinyl alcohol, polyvinyl pyrollidone, or similar compounds. The binders and their amounts might be changed, with the sorbent being made into a plate to ensure a better product able to withstand the mobile phases most used with that particular sorbent. When these plates are made, oven drying (rather than air drying) is routine, so the plates from a newly opened box are fairly active.

One possible complaint with the polymer-bound plates is the softening and swelling that occur with certain solvent combinations. In the worst case, the layer can wrinkle or lift off the support. Often, on questioning people who have experienced this, it is found that they did not activate their plates. Although this is routinely done to dry the TLC plate to give greater reproducibility, it has a second positive effect. The additional drying can also help increase the binder strength. Presumably, this occurs because the heat causes extra cross-linking of the binder and/or the removal of water.

A final solution to the lifting or softening of these layers is to use 1 M sodium chloride in place of the water portion of the mobile phase in polymer-bound RP plates. The salt prevents hydration of the binder so that swelling or buckling is much less likely to occur.

If these additional steps do not help, then it is advisable to change to a plate designed to be used with a particular mobile phase. Many manufacturers have produced TLC plates to be used with highly aqueous developing solvents, because their original prepared layers could be a problem with such developing solvents.