

duction of close-fitting pieces of PTFE. The compartments are filled simultaneously to equal height with suspensions of different adsorbents. The plates are coated in the usual way.

Impregnation gradients are usually obtained by immersing a chromatographic plate for a moment in a solution of the impregnation agent or by suspending the adsorbent in a solution of the impregnation agent and simultaneously spreading the different suspensions on the plate (3,31).

Stahl (33,34) described an apparatus for obtaining continuous stationary-phase gradients that maintained the basic construction principle of the normal spreader. A rectangular case divided diagonally into two compartments by a partition wall is filled with two different adsorbent suspensions. When the sliding bottom of the case is opened, the suspensions fall into the spreader cylinder, which is divided into several small compartments, and mix in various proportions. After mixing of the compartments' contents, the plates are coated in the usual way (for details see Refs. 3, 6, 31, 33, and 34).

Activity gradients on adsorbent layers are very convenient (48,49). The Vario-KS chamber permits preadsorption of vapors on the adsorbent layer, which is placed face down over a tray that contains various solvents. The removable tray consists of many rectangular troughs that can be filled with different solvents or humidity-controlling liquids (details are in Ref. 49). The eluent is in a separate trough and can be delivered to the adsorbent layer by a wick.

### III. GRADIENT ELUTION

#### A. Polyzoal Thin-Layer Chromatography

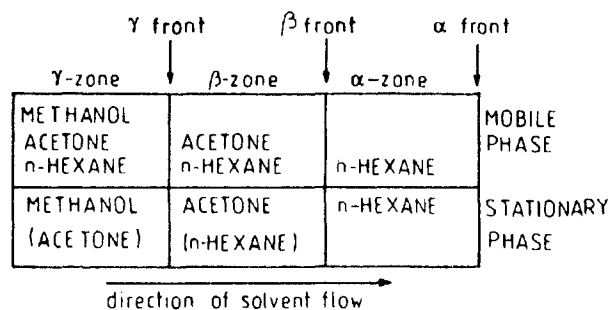
Polyzoal TLC (6,7) can be carried out only in a cooled sandwich chamber. Experience has shown that the phenomenon of solvent demixing can take place mainly in sandwich chambers. If a binary mobile phase migrates through an adsorbent layer, e.g., silica gel, the molecules of stronger solvent are preferentially adsorbed, resulting in demixing of the mobile phase. This effect is the basis of frontal analysis and polyzoal chromatography (6,7). The demixing effect is more pronounced when a cooled sandwich chamber is used (for example, a Brenner-Niederwieser chamber). The demixing effect is also more pronounced if the components of the mobile phase differ strongly in eluent strength.

When the solvent molecules are selectively adsorbed during the separation process and solvent demixing occurs, the  $\alpha$  zone, containing only the weak solvent, is formed. Behind the  $\alpha$  zone, the  $\beta$  zone, containing in the stationary phase the molecules of the stronger solvent, is formed. The  $\beta$  zone is separated from its predecessor by the  $\beta$  front. Zone and front formation with a ternary mobile phase are illustrated in Fig. 6.

The migration rates of the fronts are different and can be expressed by the retardation factor

$$k_{\beta} = \frac{\text{Distance from immersion line to } \beta \text{ front}}{\text{Distance from immersion line to } \alpha \text{ front}}$$

The  $k_{\beta}$  factor for a given adsorbent and mixed eluent is a function of the concentrations of



**Figure 6** Phase formation with multicomponent solvents (polyzoal TLC) in an unsaturated sandwich chamber.