component are uncorrelated with those for the first principal components. The variance of the third principal component is smaller than those of the first two principal components, but it is higher than the variances corresponding to the next components.

It is theoretically possible to determine *n* principal components. The question is, how many factors do we want to extract? Because they are obtained in order of decreasing contribution to the total variance and they account for less and less variability, it is usually sufficient to consider the first few principal components that still retain most of the variance. The decision as to when to stop basically depends on when there is only a very little random variability left. This decision is arbitrary, but several methods have been proposed for making it. One much used method is to select the first *p* principal components in such a way that they account for at least 80–90% of the total variance. Another criterion often used to select principal components is to keep eigenvalues that exceed 1. In practice, two or three principal components usually account for an important part of the variance.

The loadings corresponding to the principal components are plotted (Figs. 8 and 9), with each variable represented as a point. From this plot, it can be seen which of the initial variables have the greatest shares in the variance of particular principal components. Furthermore, scores plots are very useful as a display tool for examining the relationships between objects and looking for trends, groupings, or outliers (73).

An example of the application of PCA to the choice of optimum solvent system is the paper of Bota (74), who used this method to find the optimum mobile phase for the separation of seven polycyclic aromatic hydrocarbons. They concluded that the PCA enables rational selection of a restricted set from nine available mobile-phase systems and is a useful graphical tool.

IV. AUTOMATED MULTIPLE DEVELOPMENT

Complex mixtures containing components with a wide range of polarities or molecular structures cannot be separated by isocratic TLC. Low-strength solvents will separate the compounds weakly retained on the stationary phase (high R_f values), while the compounds strongly retained on the layer (low R_f values) will migrate short distances. On the other hand, strong solvents cannot separate the poorly retained compounds, which migrate as a single spot or as unresolved spots. Automated multiple development (AMD) (75–77) is used to solve these problems so that optimum separation will be achieved. AMD is a sequential, programmed, incremental, multiple development technique using, for silica gel, a gradient of the mobile phase starting with a very polar solvent, decreasing the polarity of the mobile phase with a solvent of medium polarity, and ending with a nonpolar solvent.

The mobile-phase gradients are generated step by step using as many solvents as necessary to realize the desired separation. The number of steps is kept as low as possible to optimize the

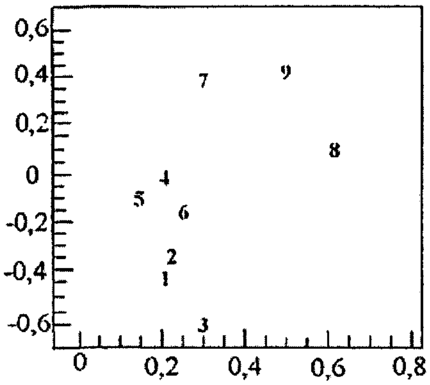

Figure 8 Plot of the first two loading vectors (A_1 and A_2). (From Ref. 74.)