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Note

Effect of degree of coating on column efficiency in liquid chromatography

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Silica gel which is physically coated with a solid substance is still a useful stationary phase in liquid chromatography (LC), in spite of the high performance of other LC systems. Such coated systems have been shown to be advantageous for the separation of geometric[1] and optical isomers[2,3]; transition-metal complexes[4,5] and charge-transfer acceptors for complexation with polyaromatic hydrocarbons (PAHs)[1–3] are typical examples.

In situ[3,4] coating is a generally accepted method, but this technique possesses intrinsic problems, such as inhomogeneity of the coating and uncertainty of the amount of the coating material. In the course of high-performance liquid chromatography studies on charge-transfer complexation between biological compounds with PAHs we observed that the amount of coating on silica gel seriously affects the column performance.

We report here the influence of the amount of riboflavin (vitamin B_2), the coating material, on silica gel for PAH separation.

EXPERIMENTAL

The experimental procedure used for the preparation of the high-performance column has been reported[6]. Since the purpose of this study was to examine the effect of the amount of coating on the performance of the column and not to optimize the conditions, an inexpensive silica gel was employed. A known amount of Partisil 20 (Whatman, Maidstone, Great Britain) was added to an aqueous solution containing a known amount of riboflavin. Water was evaporated slowly at 80°C using a rotary evaporator. Coating was complete in 2–3 h. The coated silica gel was finally dried in high vacuum (1.0 mmHg) at 80°C overnight. A 25 $\times$ 0.21 cm I.D. stainless-steel column was prepared by the dry-packing method. In order to ensure comparable conditions, the same stainless-steel tube was used for the different stationary phases. A Waters 6000 pump, Reodyne 2710 injector, and LDC UV detector (254 mm) were employed.

RESULTS AND DISCUSSION

Naphthalene, phenanthrene, and pyrene were taken as a standard mixture in methylene chloride solution. Silica gel columns which were coated with 0%, 2%, 5%,

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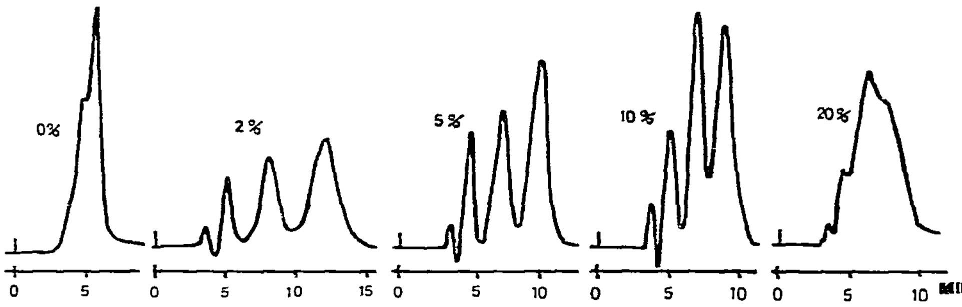

Fig. 1. Separation of PAHs on a riboflavin-coated column. The peaks are naphthalene, phenanthrene and pyrene, in order of increasing retention time. Percentages above the chromatogram indicate coating amount. Column dimensions, 25 × 0.21 cm I.D.; eluent, 10% CH_2Cl_2–*n*-hexane; flow-rate, 0.5 ml/min.

10% and 20% (w/w) riboflavin were prepared. The chromatograms of the standard mixture on these columns are shown in Fig. 1. The striking effect of riboflavin on the separation can be seen by comparing the chromatogram obtained with the uncoated silica gel column and the 2% coated column. The column performance starts to deteriorate when the amount of coating material is more than 5%. The retention time also changes according to the coating amount.

The effective molecular area of riboflavin on silica gel was calculated to be 7.4 nm^2, according to Snyder[7]. Assuming the surface area of the silica gel to be 400 m^2/g, *ca.* 3.3% coating should provide a monolayer coating. Apparently, exceeding this amount does not contribute appreciably to the retention, but it disturbs mass transfer by blocking the pores of the silica gel. Furthermore, the surface area of the coated silica gel is decreased when the coating material is coated as a multilayer. This argument may explain why both performance and retention decrease with increasing amounts of coating material on the silica gel. It seems, however, that since a true monolayer coating is difficult to achieve, the reproducibility of the column in this range is problematic. For column reproducibility, probably somewhat more than a monolayer coating is preferential, as long as the column performance is satisfactory.

This study suggests that when coated silica gel is employed for separation, the surface area and pore size of the silica gel, as well as the effective molecular size of coating material, dictate the amount of coating required for optimal separation conditions.

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