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APPLICATION NOTE
POLYAMIDE 6 FOR TLC

Separation Mechanism For Polyamide

The polyamide thin layer coating consists of small particles that are, however, much larger than the eluent or substance molecules. On the surface and within the particles there are functional groups, i.e. peptide groups and – in much smaller number – terminal amino and carboxyl groups.

The eluent flows through the cavities between the particles. Between the eluent molecules and the functional groups of the polyamide different forces can appear depending on the types of molecules and groups involved. These include ionic, dipole, electron donor/electron acceptor, and hydrogen donor/hydrogen acceptor interactions.

The hydrogen donor/hydrogen acceptor interactions, which lead to the formation of hydrogen bonds, play an important role due to the nature of the functional groups on the polyamide. During chromatography, the eluent molecules will first encounter the functional groups on the surface of the particles and will saturate these depending on the types of interactions involved. More eluent molecules will bind to these centers until finally a relatively stationary liquid film is formed on the surface of the particle.

Also, molecules from this liquid film can reach the interior of the particle depending on their structure and polarity. There they can saturate more functional groups. Hydrogen bonds between the polymer chains are broken up. More eluent molecules can accumulate at these centers.

The result is that the polyamide swells and under extreme conditions may be dissolved. Thus, in polyamide chromatography, the stationary phase consists of a liquid film on the surface and a swollen layer inside the particle, provided, naturally, that the interactions between the eluent molecules and the functional groups are not too low.

During chromatography, the substances to be separated will be distributed between the resulting stationary phase and the eluent flowing between the particles and the mobile phase. Simultaneously, however, substances compete in an adsorption/desorption reaction with the eluent molecules, which saturate the polyamide, for positions on the adsorbent polyamide.

One must consider two superposing effects which will influence the separation: the type of eluent as well as on the type of substance to be separated. Due to many unknowns, no quantitative predictions for Rf values are possible.
However, the considerations mentioned above allow certain semi-quantitative information about the relative position of substances in different eluents and hence, confine the number of possible eluent systems for a certain separation problem from the beginning.

The special character of the separation processes on polyamide causes the same fundamental advantages compared to other adsorbents. Due to its swelling properties polyamide has a high adsorption capacity. Thus, it is suited for preparative chromatography as well as for the analysis of biological material (separation of undesirable accompanying compounds).

Often polyamide chromatography is applied for the isolation and identification of natural substances with phenolic and polyphenolic groups, e.g. anthocyanins, anthoxanthines, anthraquinone derivatives, and flavones. Via modification of adsorption and partition effects, the chromatography on polyamide can be varied substantially.

Due to the medium polarity of polyamide, the stationary phase (polyamide swollen with eluent) can be made polar than the mobile phase. Consequently, it is possible to develop in two dimensions with normal and reversed-phase. Since adsorption depends not only on the number and the activity of the polar groups but also on the sterical shape of the substances, structure isomeric compounds can be well separated on polyamide.

Detection Reactions on Polyamide Chromatograms

Polyamide as organic amide is not stable towards aggressive visualization reagents (acids and bases), therefore with polyamide non-destructive detection under UV light is preferred viewing under short- or long-wave UV light (native fluorescence of substances to be visualized).

This method is especially suited for phenolic and polyphenolic natural substances; fluorescence quenching (absorption) caused by the substances in question on a fluorescent plate; visualization under UV light after spraying with special reagents.