the van Urk reaction with lysergic acid derivatives, or an ammonia solution or ammonia vapor can be employed to stabilize the reaction of tryptamine with 2,6-dibromoquinone-4-chloroimide. For fluorescent zones, the chromatogram plate is treated with a viscous lipophilic or hydrophilic agent. These agents evidently influence the rotation of the molecules and keep out the ambient air to help eliminate quenching. As lipophilic stabilization agents, particularly liquid paraffin, but also silicone, kerosene, isooctane, or dodecane are used at low concentrations. Often, more concentrated solutions additionally yield an intensification of the fluorescence. As hydrophobic stabilization agents, for example, polyethylene, triethylamine, triethanolamine, or Triton X-100 are used.

C. Bioactivity-Based Detection Methods

Microbiological and biochemical methods of detection do not exploit chemical or physical properties but the biochemical or biological–physiological activity of substances. Bioactivity-based reactions are employed mostly for the detection and determination of environmental or toxic compounds such as pesticides (insecticides, fungicides, herbicides), antibiotics, alkaloids, mycotoxins, cytotoxins, hot or bitter substances, and saponins. Such compounds have in common that they stimulate or inhibit an appropriate enzyme or test organism during incubation. Either enzymes or test organisms can be applied directly onto the sorbent layer (bioautographic detections) or the sorbent layer itself is placed on the test organism medium (reprint methods). For biochemical detection, enzymes are used. Appropriate test organisms for microbiological detection can be mold spores, yeast cells, cell organelles, or bacteria in a nutrient medium.

For instance, saponins are detected by blood cells. After chromatography, a blood–gelatin suspension is directly applied onto the layer. Then active agents diffuse from the layer to the blood–gelatin suspension and stimulate or inhibit the test organism during incubation. Saponins cause hemolysis of blood cells, so they are visible as transparent, nearly colorless zones on a turbid red blood–gelatin background.

Antibiotics in environmental samples can be detected by the bacterium *Bacillus subtilis*. The plate is dipped in the bacterial solution. After incubation, the plate is sprayed with MTT–tetrazolium salt reagent, which, after incubation, gives a blue-violet background. Antibiotics inhibit the growth of the bacteria and cause bright zones of inhibition on the colored background (Merck Chrom Biodip®, bioautography test kit).

The principle of enzymatic reactions is the formation of an enzyme–substrate reaction (32). The developed chromatogram is dipped in an enzymatic solution, e.g., a solution of cholinesterase, and incubated for a short period. Then it is dipped into a substrate solution, e.g., 1-naphthyl acetate/Fast blue salt B. In presence of the active enzyme, 1-naphthyl acetate is hydrolyzed to 1-naphthol and acetic acid. Further, 1-naphthol is coupled with Fast blue salt B to form a violet-blue azo dye. This enzyme–substrate reaction is inhibited by pesticides, such as organophosphates, organochlorines, carbamates, or pentachlorophenol, which inhibit the enzyme cholinesterase. Consequently, such substances cause bright zones of inhibition on a violet-blue background (33,34). Also, other enzyme test systems, such as (chymo)trypsin, elastase, urease, amylase, aminolevulinic acid dehydratase, vegetable peroxidase, or catalase can be applied.

Advantages of bioactivity-based detection are

1. High specificity and reduced interference of the matrix, leading to a reduced need for sample cleanup.
2. More sensitive detection limits, i.e., typical detection limits are found to be in the subnanogram and even in the lower picogram range.
3. Coupling of chromatography with bioactivity, allowing identification of toxic compounds, degradation products, or metabolites, not just the summation of damaging effects in a specified test system as in biomonitoring tests. Separated fractions are stored in the chromatogram and can easily be used for bioactivity-based reactions.

Coupling of bioactivity detection with TLC enables the assignment of physically detected substances to a specific activity, which means that toxic active substances can be identified, not just