III. TLC OF ANTIBIOTICS—OTHER APPLICATIONS

Apart from antibiotic analysis focused on the separation of antibiotics of one or different classes, there are many other examples of TLC applications. Some of them are described in this section.

A. Purification of Newly Discovered Antibiotics

Antimicrobial substances produced by *Bacillus subtilis* BS 107 were isolated from culture filtrate by precipitation and extraction. They were then purified by TLC on silica gel plates developed with ethanol–water mixture (2:1) (126). The AH7 antibiotic produced by *Streptosporangium roseum* strain 214 was extracted with chloroform from the filtrate culture and purified using TLC (127). A mixture of polypeptides was isolated from the culture broths of the mold *Stibella flavipes*. Using preparative TLC, three groups of peptides—stilboflavins A, B, and C—could be separated (128).

B. Biomolecular-Chemical Screening

Chemical screening can be regarded as a systematic approach in the search for new biologically active compounds in extracts from natural sources (e.g., microorganisms or plants) (129). The chromatographic parameters of microbial metabolites separated on TLC plates as well as their chemical reactivity toward staining reagents allow an almost complete picture of a secondary metabolite pattern (fingerprint) to be obtained (130). In contrast to biological screening, chemical screening is not correlated with any biological effect. Therefore, a new screening strategy, called biomolecular-chemical screening, was developed that combines the chemical screening strategy with binding behavior toward DNA (131). Pure secondary metabolites were analyzed for DNA-binding properties on silica gel RP-18 W F_{254} plates in a solvent consisting of methanol–1 M aq. ammonium acetate (4:1) (131,132). Crude extracts were analyzed by two-dimensional TLC. In the first dimension the metabolites of the extract were separated using methanol–0.5 M aq. ammonium acetate (1:3). Interactions with DNA were analyzed in the second dimension using methanol–1 M aq. ammonium acetate (4:1). DNA was spotted in a thin straight line above the separated extract before the second chromatographic step (131,133). Detection was done by means of UV extinction at 254 and 366 nm as well as by colorization with staining reagents. Changes in R_f values indicated an interaction between ligand and DNA and were expressed by the R_{f2}/R_{f1} ratio, in which R_{f1} represents the R_f value without DNA and R_{f2} represents the R_f value with DNA.

C. Examining the Stability and Breakdown Products of Antibiotics in Solutions and Dosage Forms

The stability of tetracycline in methanol solution was investigated by ultraviolet-visible (UV-Vis) spectroscopy, HPLC, and TLC (134). HPTLC analysis was done using a scientifically operated charge-coupled device (CCD) detector (135). Fluorescence detection mode was used with a 360 nm excitation source and a 550 nm bandpass filter. HPTLC plates impregnated with saturated Na_2EDTA solution were developed with methanol–dichloromethane–water (30:64:6). After separation, the plates were dried and dipped in a solution of paraffin in hexane. Compared with the standards (tetracycline, anhydrotetracycline, 4-*epi*-anhydrotetracycline, 4-*epi*-tetracycline, and chlortetracycline), more than 10 fluorescent compounds were detected from the degraded sample, in which a very small amount of tetracycline remained.

Epicillin breakdown products were demonstrated by thin-layer chromatography (136). The results were correlated with iodometric and spectrophotometric data.

Chloramphenicol monosuccinate and gentamycin as well as their degradation products were determined by TLC for quality control both of freshly produced drugs and of those stored long-term (137). Chromatography of gentamycin was done on silica with chloroform–methanol–conc. aq. ammonia (1:1:1). Chloramphenicol was analyzed on silica with *n*-butyl acetate–acetic acid (96:4).