SL17 BioArena: hyphenation of OPLC with bioautographic detection

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Recently, the resistance of microbial strains to antibiotics is a big problem in the field of anti-microbial animal and human therapy. Microbes have developed mechanisms of resistance to all classes of antibiotics available for systemic use in humans. Therefore, the research of these resistance mechanisms and the search for new antibiotics or antibiotic-like substances are actual tasks of the pharmaceutical science. The biological systems as microbes or plants contain thousands of constituents and are a valuable source of new and biologically active molecules, e.g. antibiotics or antibiotic-like substances. For their investigation, it is important to have suitable biological assays and chemical screening methods. Among the bioassays, the direct bioautography is applicable to microorganisms that can grow directly on a chromatoplate after the separation (1). There is a possibility for an advantageous combination of the layer liquid chromatography with the direct bioautography, so all the steps of the combined method (separation of the constituents, pre-conditioning, incubation, visualisation) are performed on the same sorbent layer. It is obvious that a column system is not suitable for such investigations. Among the layer liquid chromatographic techniques, over-pressured layer chromatography or optimum performance laminar chromatography (in short form: OPLC) integrates the advantages of the conventional TLC/HPTLC and HPLC. The combination of bioautography with the automated OPLC results in the so-called BioArena (2) as a complex bioautography system which exploits attractively the advantages of OPLC giving compact spots and good resolution and sensitivity. This system provided optimum conditions for the detection of ingredients from grapes, cabbage and paprika as unique medicinal plants. However, BioArena generates also other advantageous features. It can be used for studying the role of changeable incubation time in the mechanism of action of antibiotics and the interactions between the microbes and the dye substance as well as other small and big molecules as co-factors in the sorbent bed after OPLC separation.

References: 1. Botz,L., et al. (2001) In: Planar Chromatography (ed.: Sz. Nyiredy) , p. 489, Springer Budapest. **2.** Tyihák, E. et al. (2001), In: Proc. Planar Chromatography, Lillafüred, Hungary, p. 3.

SL18 Biosensoric detection of the cysteine sulphoxide alliin

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Garlic (*Allium sativum* L.) and related species of the Alliaceae family are known for their cancer-protecting and antiatherosclerotic potential. Sulphur containing flavour compounds are responsible for the characteristic smell and taste of members of this family. These volatile flavour substances are formed by the action of alliinase (EC 4.4.1.4) on cysteine derivatives, when plant material is disrupted (1). Intact bulbs contain mainly the odourless, nonvolatile precursors such as (+)-S-(2-propenyl)-L-cysteine sulphoxide (alliin).

In the present investigation, an alliin-specific biosensor exploiting immobilized alliinase has been developed. Besides volatile compounds like allicin, also pH-active substances as pyruvic acid and ammonia were formed, which can be detected by a pH sensitive electrode (2, 3). Enzymically formed ammonia was detected either by a potentiometric sensor based on an ammonia electrode or a pH-sensitive electrolyte / insulator / semiconductor (EIS) layer structure made of Al/p-Si/SiO_2/Si_3N_4. It could be demonstrated with both methods that this biosensoric method yielded results comparable to sensitivities obtained by HPLC. Alliin concentrations down to $6x10^{-6}$ M could be quantified.

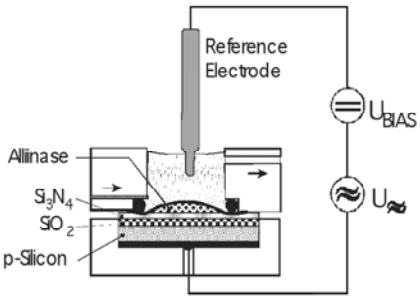

Figure Experimental set-up of the alliin biosensor (EIS). The cavity above the alliinase-layer contains the sample solution.

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