Chapter 1 : Formats and Editions of Analytical and preparative isotachophoresis : proceedings [www.nxgvi

Analytical isotachophoresis in polyacrylamide gels was used to develop a high resolution system to separate the components of histo-plasmin. The analytical system was then expanded to preparative-scale isolation of the skin test-active components of histoplasmin.

Previous Section Next Section Introduction Lipoproteins may be classified according to their density, particle size, electrophoretic mobility, or apolipoprotein composition 1 â€" 5. It has become obvious that among the major lipoproteins, and particularly among HDL, there are functionally distinct particle subclasses. Various methods have been developed to characterize HDL subclasses, such as ultracentrifugation, agarose gel electrophoresis, gradient gel electrophoresis, chromatographic methods, and immunoaffinity chromatography 6 â€" One of the most interesting approaches is two-dimensional gradient gel electrophoresis 2D-GGE followed by immunoblotting for apoA-I. A major drawback of 2D-GGE is its poor suitability for preparative purposes. Another method for separation of lipoproteins by their electrophoretic mobility is capillary isotachophoresis, which is a support-free technique with negligible molecular sieve effects. It is performed in a discontinuous buffer system with the advantages of concentration and self-sharpening effects. With the introduction of NBD-ceramide by our group, the first quantitative label of lipoproteins became available ITP can be automated for the analysis of large sample series. So far, analytical ITP protocols could not be transferred to a preparative scale. Therefore, it was impossible to fully characterize the subfractions observed by analytical ITP. The latter method has the drawback that steady state is not reached when the number of sample components increases and only small differences in electrophoretic mobility exist. Therefore, ultracentrifugally prepared lipoproteins and not total plasma had to be used in these protocols. In a recent paper based on our methodology Schlenck et al. They show that the subfractions change specifically with changes in lipoprotein concentrations or composition. This was taken as an indication that analytical ITP may be useful for diagnostic purposes. We recently developed an automated, preparative free solution isotachophoresis FS-ITP system suitable for separation of total plasma proteins in the milligram range FS-ITP separates lipoproteins from whole plasma in the batch mode without the problems inherent to continuous flow systems. These data will provide the basis for a broader use of analytical ITP as a method for lipoprotein analysis. Capillary isotachophoresis of lipoproteins Analytical capillary ITP of plasma samples was performed as described earlier 23 with minor modifications. Plasma was diluted 1: The terminating electrolyte contained 20 mm alanine and was adjusted to pH 9. Samples were injected into the capillary using pressurized injection for 18 s at 3. The separated zones were monitored with laser-induced fluorescence detection excitation nm; emission nm. Preparative isotachophoresis of lipoproteins Preparative separation of lipoproteins was per formed as recently described 27 , with minor modifications for the separation of lipoproteins. The leading chamber buffer contained 10 mm HCl and was adjusted with ammediol to pH 8. Both buffers were prepared in 0. The fractionation buffer and leading electrode buffer consisted of 10 mm HCl adjusted with ammediol to pH 8. The terminating electrode buffer was 40 mm alanine adjusted with Ba OH 2 to pH 9. The sample was injected with a 1-ml syringe and the separation was started with constant current of 2 mA. The voltage remained constant for the first 10 min at V and increased within 80 min to V. The current was then reduced to 1 mA and the voltage decreased to V. After a further 5 min the line of the added marker dye was 1 mm in front of the fractionation channel and the voltage had reached a value of V. This value was adjusted at the interface, as the signal to start collecting fractions. The dye line moved with an effective velocity of 1. After min the separation was stopped. The voltage had reached a constant final value of V. Molecular mass standards Pharmacia, Freiburg, Germany containing thyroglobulin, kDa; ferritin, kDa; catalase, kDa; lactate dehydrogenase, kDa; and albumin, 67 kDa were run simultaneously with the sample. Immunoblotting and chemiluminescent detection After electrophoresis, proteins were electrophoretically transferred to fluorotrans transfer membranes of 0. The lane with molecular mass standards was cut off and stained with Coomassie Blue. For the detection of the individual apolipoproteins, membranes were incubated for 1 h with the corresponding antibodies in a dilution of 1: The following antibodies were used: After antibody incubation,

the membrane was washed three times 10 min each in 50 ml PBS containing 0. Before chemiluminescent detection the washing step was repeated. The second immunodetection was performed as outlined above. Total and free cholesterol were determined by a cholesterol oxidase assay with fluorimetric detection. For the determination of free cholesterol, no cholesterol esterase was added to the incubation. Concentrations of enzymes and detergents relative to cholesterol were chosen as described 31 , ApoA-I was determined by a sandwich type enzyme-linked immunosorbent assay according to Koren et al. ApoA-IV concentrations were determined by a previously described sandwich type enzyme-linked immunosorbent assay Quantification of apoD was performed by an immunoassay with time-resolved fluorescence spectroscopy A polyclonal rabbit anti-apoB antiserum Behring or a polyclonal goat anti-apoE affinity-purified IgG fraction Biodesign International , respectively, were used for coating. A reference standard for human apolipoproteins Immuno, Vienna, Austria was used for calibration. Immunoblotting and chemiluminescent detection were performed as outlined above. For the relative distribution of apoC-III, The relative concentrations were calculated from calibrated intensity values Boehringer Light Units, BLUs as described by the manufacturer. Lipoproteins containing apoA-I LpA-I as the only apolipoprotein were determined by an electroimmundiffusion technique in agarose gels using kits from Sebia Issy-les-Moulineaux, France. By addition of purified lipoproteins, these fractions can be further identified: When the same sample is separated repeatedly and the peak areas in the HDL range peaks 1â€”3 are determined, there is a between-run coefficient of variation CV of 4. It is apparent that there are specific changes in the HDL region associated with certain diseases or conditions. The fractionation begins with appearance of the added marker dye. The latter was assessed by performing 6 separations on 2 different days from the same sample. The CV was 5. When only single fractions were analyzed, the CV was 3. The distribution of apoA-I-containing lipoproteins was determined after electrophoresis through 0.