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Buffer preservative failure and modification of in-line buffer filter for automated step-change amino acid analyzers

One of the continuing problems in operation of automated liquid ion-exchange amino acid analyzers is microbial contamination of the buffers. Failure of the usually recommended preservatives, octanoic acid and pentachlorophenol, apparently is much more common than generally recognized or admitted. Moreover, problems attributed to other things, such as entrapped air, careless buffer preparation, or insufficiently "cleaned" sample, may in fact be unrecognized microbial contamination. Troubles have appeared with either sodium or lithium citrate buffers above about pH 4. The higher the pH, the more likely the difficulties.

Because of short column length for basic amino acids and very short running time, difficulties are encountered less frequently in two-column analyses of simple protein hydrolysates. However, serious problems have arisen with extended runs on longer columns, as required for complex mixtures such as physiological fluids or plant or soil extracts. For example, before signs of fungal growth were ever visible in our system, excessive column back-pressure (> 500 p.s.i.) developed in the basics column (0.9 × 27 cm PA-35 resin) which was ultimately traced to blockage of the resin support screen and/or teflon sponge at the bottom of the column. In one instance (unattended night run), pressures exceeded connector clamp strength and the whole bed of expensive spherical bead resin was pumped out and lost. This was baffling since blockage is usually at the top of or within the column, which results in abnormal compression or apparent shrinkage of the resin leading in the extreme to failure of top rather than bottom of column connections. Subsequent staining tests and microscopic examination showed contaminant material was passing the full length of the resin bed only to lodge in the resin support disc. Fortunately, regenerating hydroxide removed enough contaminant to permit a series of runs but resistant residuals did accumulate; this necessitated periodic replacement of the clogged disc. The problems were accentuated by changing to a three-buffer single-column (0.9 × 60 cm) routine for acidic, neutral and basic amino acids.

Increased dosages of octanoic acid and pentachlorophenol were ineffective or interfering. Other preservatives at initially effective concentrations appear either no better, alter sequence of elution, degrade resolution or otherwise are incompatible. MARAVALHAS[1] recently suggested diethylcarbonate as a more useful preservative which acts "...fairly well" under his conditions in South America. It remains to be seen how effective this chemical will prove for others, or how long it will remain effective before tolerant or adaptive organisms develop, as they have for octanoic acid and pentachlorophenol.

Meanwhile, analyzer owners interpose a variety of in-line filters just ahead of or in the column top connector. An effective unit for high-pressure, high-flow rate systems is the "micule filter"* (available through Beckman-Spinco, Palo Alto, Calif., U.S.A.). This is in effect a miniature column (0.9 cm I.D.) containing about 1-cm depth of inert resin beads (of essentially the same particle diameter as used in the analytical

* Mention of trademark or company names is for identification purposes and does not constitute preferential endorsement by the U.S. Department of Agriculture.

column) sandwiched between two teflon sponge disks. This inert resin supposedly is the essential part of the filter for removing all particulates, including fungal contaminants. In our system, the porous teflon sponge supports are actually the effective filters. The special resin is not needed, thereby saving both the cost and the fuss of removal, clean-up and replacement of the resin. In its place, we have simply added additional teflon sponges—as a convenience and to minimize mixing or buffer hold-up volume. When excessive back-pressure develops, the top sponge is removed; a clean disk may then be inserted, preferably at the bottom.

The teflon sponge disks are the same as those regularly used for resin support in the analytical column, and are commercially available. Alternatively, extra disks may be cut to snug-fit size, with an ordinary sharp cork borer, from a $\frac{1}{8}$-in.-thick sheet of 50–55% nominal porosity teflon sponge. New or clogged disks are cooked in refluxing concentrated nitric acid for several hours until pure white (overnight is convenient and insures complete cleaning). We might add that our sheet of stock teflon was variegated gray rather than the typical white. Microscopic examination showed the grayish color was from charcoal-like particulates in the teflon. The HNO_3 treatments removed these. They also disappeared after baking in a muffle-furnace at $> 600°$ (suggestion from Dr. P. B. HAMILTON, Dupont Institute, Wilmington, Del., U.S.A.). Clogged disks from the buffer filter probably could be adequately cleaned by similar "baking", provided the teflon sponge withstands such repeated heat treatments.

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