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Tetramethylbenzidine

A reported noncancerogenic analog of benzidine

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{Me} \\
\text{Me} & \quad \text{Me} \\
\text{Me} \quad \text{NH}_2 & \quad \text{Me}
\end{align*}
\]

For many years benzidine was used as a sensitive and specific reagent for the detection of blood. However, in 1974 the Occupational Safety and Health Administration banned its manufacture and use in the United States because of its extreme carcinogenicity.

One of the early hypotheses for the carcinogenicity of aromatic amines involved ortho hydroxylation. Thus, it seemed that 3,3',5,5'-tetramethylbenzidine (TMB) (in which ortho hydroxylation is impossible) might be an effective and safe substitute for benzidine. Indeed, it was found to be nontumorigenic by subcutaneous injection into rats.

The Salmonella/microsome test (Ames test) showed TMB to be nonmutagenic, suggesting noncarcinogenicity. Recently, TMB was shown to be nonmutagenic when compared to other benzidine compounds in a modified Ames assay.

Garner et al. evaluated the use of TMB as a presumptive test for blood in forensic work. In various concentrations of glacial acetic acid, TMB reacted with blood in the presence of H₂O₂ to form a colored product. Comparative studies with benzidine showed TMB to be equally sensitive in blood detection. Piekko and Boemer have used TMB in a mixture spread on polyester film strips to determine glucose concentration in whole blood.

Standefer and Vanderjagt found that methods employing TMB for plasma hemoglobin assay compared well with those using benzidine in accuracy, precision and sensitivity. Iron porphyrins, which can exhibit a peroxidase-like action, were detected at very low levels on paper chromatograms with TMB as the visualizing agent. The peroxidase activity of the heme protein cytochrome P-450 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with TMB staining. In this method, TMB-H₂O₂ stain is superior to benzidine-H₂O₂. The blue-stained bands are distinct and the color is stable. Peroxidase activity was also detected by the presence of immunoprecipitates in Ouchterlony double-diffusion plates.

In the area of neurohistochemistry, TMB proved more sensitive than 3,3'-diaminobenzidine in demonstrating retrograde and anterograde axonal transport of horseradish peroxidase (HRP) in rat brain by light microscopy. TMB also gave results comparable to those obtained with benzidine dihydrochloride in the demonstration of retrograde transport of HRP.

Current literature is replete with references to analytical applications of TMB. Recent applications include the use of TMB in a substrate to provide direct evidence of peroxidase activity in Salmonella typhimurium, of horseradish peroxidase on virally infected plant root extracts, and to measure the activity of peroxidase in an HRP-IgE complex. TMB is gaining recognition as a chromogen in enzyme immunoassay systems.

We also offer TMB dihydrochloride which, in contrast to the water-insoluble free base, dissolves slowly in water or in citrate buffer. TMB dihydrochloride appears to be suitable for the quantitative determination of hemoglobin in solutions, and for the identification and localization of myeloperoxidase in cells.

TMB and TMB dihydrochloride have earned acceptance as reliable and safe substitutes for benzidine and its carcinogenic derivatives.

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