

**IN VIVO STAIN COMPOSITION, PROCESS OF MANUFACTURE,
AND METHODS OF USE TO IDENTIFY DYSPLASTIC TISSUE**

ABSTRACT

The N-demethylation and N,N-demethylation derivatives of the two conformational isomers toluidine blue O ("TBO") have been synthesized, isolated and identified.

An in vivo biological stain composition includes the conformational isomers of TBO and these N- and N,N-demethylation derivatives. The ratio of these isomers to these demethylation derivatives is at least 6:1.

An improved method for detecting dysplastic oral tissue includes application of such TBO products, in a liquid carrier, to oral tissue.

A process for preparing TBO products includes (1) oxidizing N,N-dimethyl-p-phenylenediamine to form an intermediate 2-amino-5-dimethylaminophenyl thiosulfonic acid, (2) oxidizing this intermediate and condensing the oxidizate with o-toluidine, forming indamine thiosulfonic acid and (3) further oxidizing the indamine intermediate to form a TBO-containing reaction product, which is precipitated from the reaction mixture as a complex, from which the final TBO product is separated. The complexing agent is added to the reaction mixture at least before the final oxidation step, preferably before the second oxidation step.

An HPLC method for characterizing TBO products

containing the conformational isomers of TBO and their - and N,N- demethylation derivatives. The a mobile phase comprises an aqueous solution of an organic acid compound.

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