

# On the Mechanism of the Neumann-Wender Glucose Test

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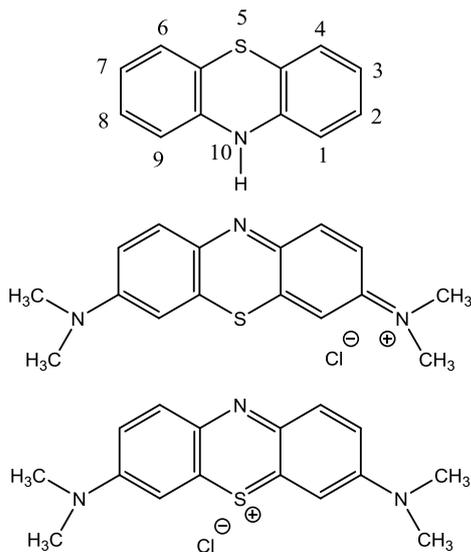
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**Abstract** This sensitive test for glucose in urine is based on the discolouration of a methylene blue solution by reaction with glucose in alkaline medium. This reaction, combined with aerial oxidation to restore the blue colour has been widely used as a showy experiment. However, the mechanism of the involved reactions has not been advanced and we provide it in this communication. The glucose enolate combines with methylene blue and then an oxido-reduction takes place by a concerted mechanism. The last reaction is a Friedemann cleavage of an alpha-ketoaldehyde into acids by means of hydrogen peroxide in alkaline medium. The mechanism of this fragmentation has been also cleared up.

**Keywords** Azanium, Degradation, Methylene blue, Oxido-reduction, Quinone-di-imine, Resonance, Thiazinium

## 1. Introduction

Methylene blue is a dye used in optical microscopy to stain nuclei of animal tissues. It is suitable as a bacterial stain and also as vital stain, i.e., can be applied on living cells without killing them [1].



**Figure 1.** Azanium chloride and phenothiazinium chloride structures for methylene blue

The frame structure of methylene blue is dibenzo[b,e][1,4] thiazine, [2]. The numbering is indicated [3,4]. Other names are thiodiphenylamine and phenothiazine. Two

structures have been advanced for methylene blue [5], Figure 1. These structures will be discussed afterwards.

The names of these structures are:

[7-(dimethylamino)phenothiazin-3-ylidene]-dimethylazanium chloride, IUPAC, and

3,7-bis(dimethylamino)-phenothiazin-5-ium chloride, CAS. In the Neumann-Wender test the use of methylene blue is proposed in order to detect the presence of glucose in urine. It is a sensitive test since 1 mL of dilute urine (1:10) is treated with methylene blue (1:1000) and Normal KOH (1 mL of each solution) and boiled for a minute. Complete discolouration indicates that glucose was present in the urine.

Since no reaction mechanism has been advanced, notwithstanding the several communications related with this reaction we provide a reaction mechanism for it, as well as for the aerial oxidation of leukomethylene blue.

The mechanism of an intervening Friedemann degradation of an alpha-ketoaldehyde by hydrogen peroxide in alkaline medium has been given for the first time.

This paper is a follow up of our studies on organic reaction mechanisms [9-13].

## 2. Antecedents

There is a brief note entitled “Methylene blue-Reduction and Oxidation”, with no formulas or references. It is only a rapid demonstration without comment or explanation, only to stimulate interest, [14].

Campbell published an article on kinetics and it is a puzzle for students in order to discover the presence of a gas in a closed flask that can restore the blue colour on shaking. No product is mentioned [15].

Other article is “Blue Bottle Experiment: Learning Chemistry without knowing the Chemicals”. It is indicated for First Year Undergraduate and General Public, a popular

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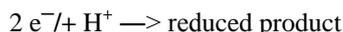
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chemical demonstration “because of its simplicity and visual appeal” [16].

As it can be seen, these are colourful presentations.

There is a communication of Anderson et al. [17] with a confusing title: “The Methylene Blue-Catalysed Air Oxidation of Glucose”. It is leukomethylene blue which is oxidized by oxygen in the alkaline medium. Although the reactants, the catalyst and reaction products are mentioned and their structures are given, there is no reaction mechanism at all. For instance, the reduction of methylene blue to the colourless compound is indicated by:



There are no free electrons in the reaction medium, stripped away from the atoms, and even less protons in an alkaline medium. The authors have forgotten the collision theory, that is, close contacts among reactive species.

In these articles the Neumann-Wender test for glucose is not mentioned at all, despite its application in Medicinal Chemistry as a qualitative analytical method. In this communication this omission has been repaired and the mechanism of the involved reactions is provided.

### 3. Discussion

As said before, two structures have been advanced for methylene blue with their respective names. But what structure represents better the chemical department of the compound? The structure with the azanium cation presents a quinone-di-imine group, an indamine [18].

The other structure, with a thiazinium, presents an o-quinone type structure. In this representation the positive charge is in the sulphur which is less electronegative (2.5) than nitrogen (3.0), [19]. Besides, this structure presents an electrophilic centre at the alpha position to the thiazinium atom, whereas the other structure does not present a reactive centre.

The structures are electromeric [20], as is shown in Figure 2.

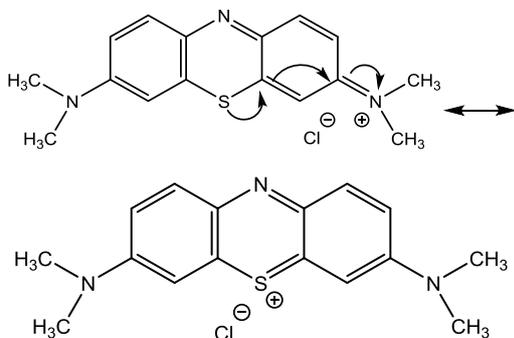


Figure 2. Methylene blue electromeric structures

In alkaline medium, glucose (a carbon acid) is ionized and forms the enolate. The first reaction step with methylene blue is the nucleophilic attack of the glucose enolate to the electrophilic centre of the cationic dye, C-12. Figure 3.

After this combination, a second enolate is formed in the

remaining glucose enol. Then a concerted reaction mechanism occurs. A ketone is formed as well as an aldehyde, and adduct is broken (oxido-reduction step). In the other fragment a cyclic sulphide has been formed and there is a concomitant electron shift from C-12 to the nitrogen due to the electron withdrawing effect of the imino group. The negative nitrogen is neutralized by reaction with water and leukomethylene blue is obtained.

The 1,2-ketoaldehyde formed is readily hydrated.

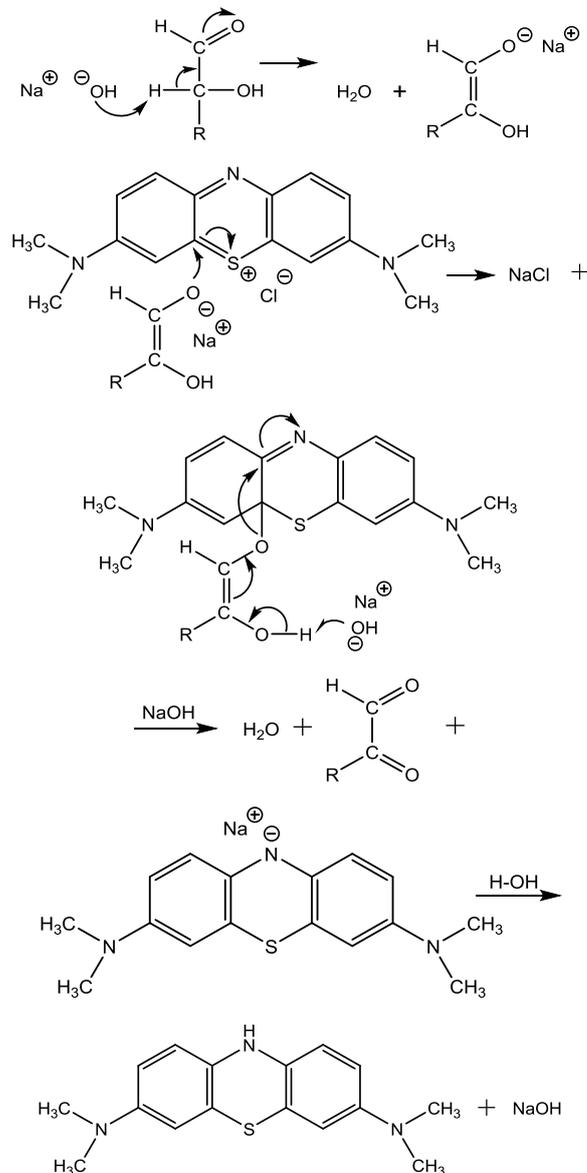
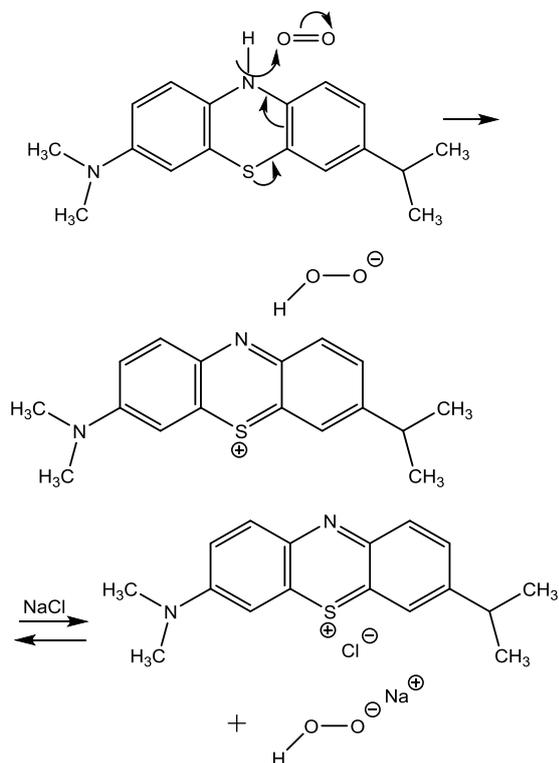


Figure 3. Redox reaction of glucose with methylene blue by a concerted mechanism

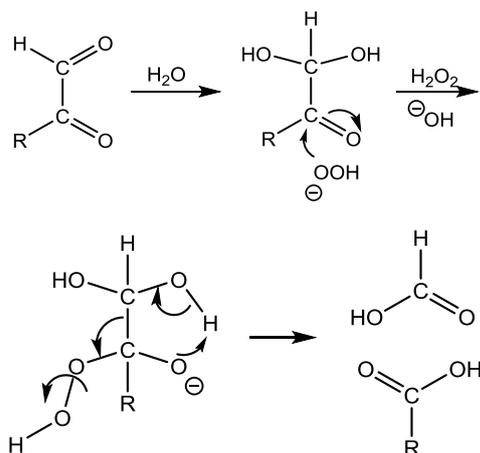
The oxidation of leukomethylene blue by oxygen absorption if the solution is shaken or swirled occurs as follows. The combined effects of an electrodotic [21] atom (sulphur) and a hydride scavenger (atmospheric oxygen), through the vinylene bridge, forms two double bonds instead of one. Now the three rings are connected by conjugated bonds, forming an o-quinone type structure, Figure 4.



**Figure 4.** Mechanism of aerial oxidation of leucomethylene blue in alkaline medium

The last stage of this reaction series is as follows: the hydroperoxide anion from the oxidation reacts with the easily formed 1,2-ketoaldehyde hydrate. The 1,1-diol blocks the electrophilic centre at C-1, in a way like a ketal, a protective group in alkaline medium.

After reaction at the keto group, the resulting alkoxide induces a proton transfer at a one bond distance in a five member ring (a 1,4-hydrogen transfer). Then a degradation reaction by a concerted mechanism occurs: formic acid results by a C-C fission, followed by a peroxide break down, yielding a pentonic acid (arabinonic), Figure 5.



**Figure 5.** Mechanism of the Friedemann cleavage of  $\alpha$ -ketoaldehydes by means of hydrogen peroxide in alkaline medium

This type of cleavage was observed by Friedemann with methylglyoxal (propanal) by the action of hydrogen

peroxide and KOH, [22, 23]. In this case formic and acetic acids were obtained.

This fragmentation reaction is related to that of Ruff [24-26]. Both methods employ an oxidized aldehyde, but at a different carbon atom.

## 4. Conclusions

The two advanced structures of methylene blue are discussed in order to choose the structure that represents better the chemical department of the compound.

The articles related to the oxido-reduction reaction between glucose and methylene blue in alkaline medium, followed by aerial oxidation, are commented.

We provide the mechanism for these reactions since there is none. The glucose enolate combines with methylene blue (adduct formation) and the oxido-reduction step takes place by a concerted mechanism of push-pull type.

The aerial oxidation of leucomethylene blue in alkaline solution occurs by a synchronous electron shift from an electron donor atom to molecular oxygen as hydride scavenger, through an intermediate double bond.

Thus, the Neumann-Wender reaction mechanism has been cleared up.

The mechanism of the Friedemann cleavage of  $\alpha$ -ketoaldehydes into acids by hydrogen peroxide and KOH has also been provided (a concerted reaction mechanism). The driving force is the push-pull effect from an alkoxide to the  $\delta^+$  oxygen at a hydroperoxide chain, involving a C—C break.

## ACKNOWLEDGEMENTS

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