American Journal of Chemistry 2021, 11(1): 18-21
DOI: 10.5923/j.chemistry.20211101.03

The Chemistry of the Weyl-Salkowski Test for Creatinine

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Abstract Creatinine is an important biomarker, thus the analytical chemistry related to it is relevant. The Weyl-Salkowski assay for creatinine is based on the reaction of this imidazolidinone derivative with sodium nitroprusside in alkaline medium, followed by acidification after the initial colours observed, being Prussian blue the final product. The fine structure of the reagent, the anionic transition metal complex, adds interest to the subject since there are two theories regarding the electron distribution in the nitrosyl-metal bond. These differences explain or not the chemical deportment of the reactants. In this communication the reaction process is cleared up. The study of this test leads to unify two theories concerning the nature of the nitrosyl-metal bond, and two alternatives are given to attain this.

Keywords Analytical chemistry, Creatinine, Reactive intermediates, Salkowski test, Sodium nitroprusside, Weyl test

1. Introduction

The Weyl-Salkowski test is based on the interaction of creatinine with sodium nitroprusside in alkaline medium, followed by acidification with acetic acid after the initial coloured steps.

Creatinine is a poly-functional compound with imidazolidine-imidazoline structures. It is a cyclic guanidine compound with a lactam group, an active methylene group, an enamine, a guanyl group and a special imido like group.

Its reactivity was studied, as well as the electron structure of the reagent, the transition metal-nitrosyl complex.

Since the chemistry of this test is unknown, we provide it and it is discussed in detail. This communication is a follow up of our studies on the chemistry of other colour tests, [1-5].

2. Antecedents

Creatinine, 2-imino-1-methylimidazolidin-4-one and its tautomer, 2-amino-1-methylimidazoline-4-one, is the anhydride of creatine, that is, a lactam obtained by cyclocondensation of creatine, N-methyl-N-guanylglycine, which is present in muscular tissue of many vertebrates, Figure 1.

Creatinine is found in the urine in small quantities, an adult excrets from 1 to 2 g per day, and it is widely distributed in plants, [6].

Creatinine is an important biomarker in order to evaluate the health of the kidneys, and it is usual laboratory blood test determine its concentration.

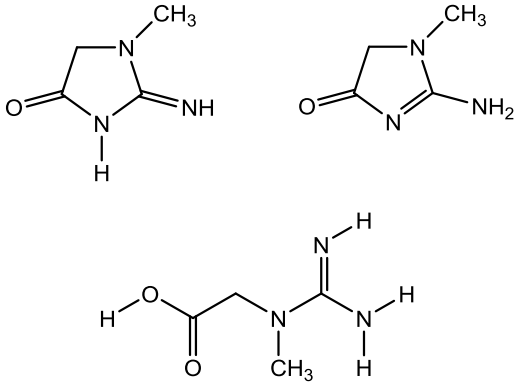

Figure 1. Creatinine tautomrs and creatine

The test for creatinine under study is due to Theodor Weyl (1851-1913). He found that a watery solution of creatinine to which a small quantity, few drops, of a solution of sodium nitroprusside has been added gives on addition of sodium hydroxide a ruby-red colour which soon changes to yellow. He published his test in Germany [7] and it was reviewed in France, [8]. The Weyl test is sensitive to the daily quantities of creatinine excreted in the urine; 5 ml of this are used.

Ernst Salkowski (1844-1923), a clinical analytical chemist, continued Weyl's assay for creatinine. If the yellow solution resulting from Weyl's test is acidified with acetic acid and then heated, the solution turns green, then blue, and Prussian blue is precipitated if much creatinine is present. He published his finding in Germany, in a physiological chemistry journal [9]. There is a biographical sketch of Salkowski, [10]. He published a laboratory manual of physiological and pathological chemistry, [11].

The Weyl-Salkowski test for creatinine has been used for

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Received: Feb. 10, 2021; Accepted: Mar. 5, 2021; Published: Mar. 15, 2021
Published online at http://journal.sapub.org/chemistry

detection of this compound in soil analysis [12], and is used also in urine medical technology, [13].

The formation of Prussian blue from the nitroprusside anion discloses the electron distribution in this anion. This will be treated in the 'Discussion'.

3. Discussion

For understanding the chemistry of the Weyl-Salkowski test for creatinine is very important clear up the electron structure of the nitroprusside anion. There are two points of view about the bond of the nitrosyl group in this metal-nitrosyl complex.

The NO ligand can be considered neutral since nitric oxide is a neutral molecule, [14]. Thus, five CN- groups and one Fe3+ ion form a 2- ferrate anion, [15]. The conception of simple coordination in the nitrosyl-metal bond is in accordance with the fact that nitroprusside releases nitric oxide, a potent vasodilator effective in the lowering of blood pressure, [16].

Pauling suggested that since nitric oxide is a free radical, this electron can be transferred to the iron atom, which is then Fe2+, and a positive charged nitrosyl results, [17]. This redox reaction can be explained by Kossel's theory: the accumulated Fe3+ positive charge diminishes, the positive charge being now in two atoms, [18].

Now let's see the reaction of creatinine in alkaline medium. Creatinine has a $–CH_2$-CO- group which can condense with aldehydes in drastic conditions, by melting the reactants in an oil bath, [19-21].

However, there is in the creatinine molecule an imido like group, with an enamine instead of a carbonyl group. The central N-H in this special group is highly reactive in alkaline medium, yielding a salt. The mentioned active methylene group is vicinal to a lactam, not to a ketone, and is less reactive compared to an imide.

Therefore, there are several reaction possibilities with the nitroprusside: hydroxyl group and creatinine salt reacting with Fe3+ and Fe2+ ferrates.

In the model of simple coordination of nitric oxide in the nitroferrate, Figure 2, **a**, the creatinine anion can displace easily the neutral nitric oxide molecule. The ligand exchange reaction accounts for a salt formation, Figure 2, **b**.

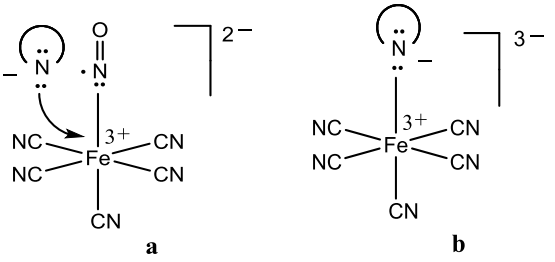

Figure 2. Halochromism in the organometallic complex

This salt can explain the red colour observed in the Weyl test, since the colour in this type of reactants is due to halochromism, that is, formation of coloured salts in strong acid or basic medium, [22].

Being the creatinine ligand negative charged, it can form a covalent bond with Fe3+, Lewis acid/base reaction. The iron is now bi-positive, there is no salt, and the colour changes to yellow, as observed in the test, Figure 3.

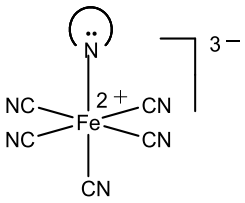

Figure 3. Creatinine with covalent bond to iron

The model in which three electrons intervene in the nitrosyl-iron bond is sustained by the fact that nitroprusside anion with Fe2+ forms in alkaline medium the $[Fe(CN)_5NO_2]4-$ anion by reaction with two -OH ions [23], Figure 4.

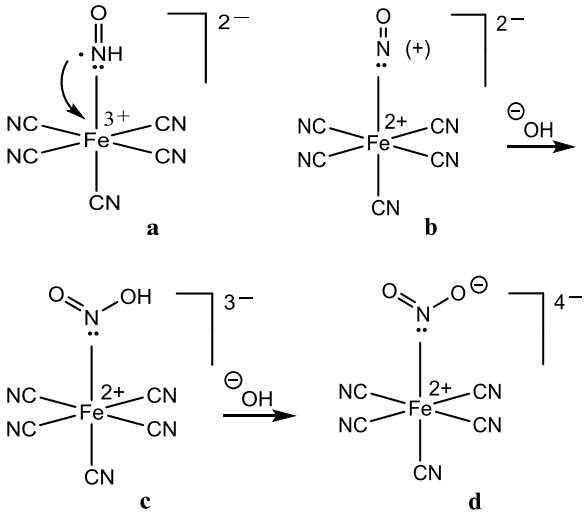

Figure 4. Formation of [Fe(CN)5NO2]4-

Exchange of the NO_2^- ligand (nitrite ion), by the negative creatinine forms another coloured salt, but a covalent bond is not feasible since there are no Fe+ ions. Therefore, the colour change from red to yellow cannot be explained as before.

Salkowski acidified the yellow solution obtained by Weyl. Protonation of the creatinine ring by acetic acid separates the heterocycle from the complex, giving the amino tautomer, 2-amino-1-methylimidazoline-4-one, Figure 5. Further protonation at N-3 yields the creatininium cation, Cf. [24].

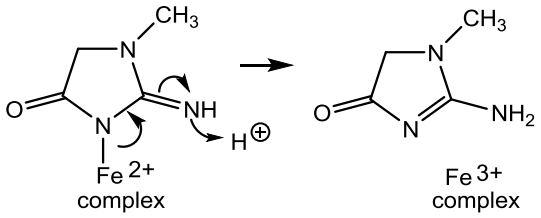

Figure 5. Detachment of creatinine from complex in Figure 3

Having been eliminated both nitric oxide and the nitrite ion, and also the creatinine, the vacant sites for coordination are filled by cyanide ions, $Fe(CN)_3$ and Prussian blue being formed, Figure 6.

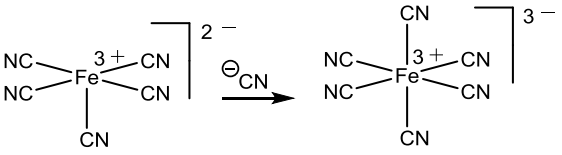

Figure 6. Formation of hexacyanoferrate(III)

Prussian blue, iron(II,III)hexacyanoferrate(III,II), requires both ferric and ferrous ions. The problem is that there are only ferric ions in the first theory or ferrous ions in the second one, so both models are needed.

A solution to two different theories, each one working only in part, is consider that there is no total transfer of the free electron in nitric oxide to the iron, this way both models remain. Other alternative is that the one electron transfer is reversible, being two convertible structures. Both possibilities can be explained by the resonance of the odd electron in the NO molecule, [25].

:N˝=Ö : ⟷ **:N̈=O':+**

4. Conclusions

The chemistry of the Weyl test for creatinine can be explained better using the neutral nitrosyl structure in the nitroprusside. The red colour comes from halochromism in an organometallic complex. The change to yellow is due to formation of a covalent bond in this molecule, instead of a salt.

Addition of acetic acid according to Salkowski removes the creatinine, neutralizing the anion and then forming creatininium ions. The vacant coordination site is filled by a cyanide group and Prussian blue is obtained.

However, the theory of a three electron bond in the nitrosyl group is needed to explain the presence of Fe2+ ions, which in addition to Fe3+ are necessary to form Prussian blue.

Therefore, two theoretical alternatives are given with the aim of unify the different interpretations about the nature and bond type of the nitrosyl ligand in sodium niroprusside.

ACKNOWLEDGEMENTS

Thanks are given to co-worker Martha Berros for support.

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