

# Trivalent Chromium Based Conversion Coating Contains Zinc/ $Zn(OH)_2$ on Iron Substrates for the Detection of Uric Acid in Biological Samples and Control of Dyeing

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Over the past decades, the researchers are using the hexavalent chromium passivation for improving the corrosion resistance of sacrificial zinc and zinc alloy electrochemical coatings on iron substrates. However, the chromium Cr (VI) is hazardous to the environment and researchers are urge to finding the replacements of the Cr (VI). Interestingly, the trivalent chrome passivation is one of the best alternative materials to date due to its unique nature such as ease of adaptability, safety and economic considerations. In this research, the factors that affect dyeing process in the trivalent chromium passivation were mainly discussed. The Zeta potential and Electrical conductivity introduced to scale the dyeing affinity. Also comparing ions between the passivation agents by IC and ICP-OES measurement to improving the intensity of coloring to achieve the optimization of the dyeing process parameters. In addition, in this work we prepared the zinc hydroxide  $Zn(OH)_2$  nanoparticles were employed to detect the uric acid (UA). Fascinatingly, the prepared sensor material shows a wider linear range of 0.02 to 154  $\mu$ M with the detection limit of 0.204  $\mu$ M and the sensor material shows the excellent found and recoveries in practical applicability of urine sample.

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**Keywords:** trivalent chromium passivation film, dyeing,  $Zn(OH)_2$  NPs, uric acid, electrochemical sensor

## 1. INTRODUCTION

Hexavalent chromium based electrolyte baths have long been used during the production of corrosion protection coatings for zinc-plated steel[1]. By dipping the zinc screw in to a chromic acid

bath at ambient temperature, a thin film consisting of a complex mixture of Cr (III) and Cr (VI) compounds is formed[2]. By varying the reaction process, the composition and parameters of the solutions the different kinds of color for the corrosion protection layer can be obtained [2, 3]. Many reports has been proved that hexavalent chromium compounds are toxic and carcinogenic [4, 5] the End-of-Life-Vehicle directive allowed a maximum of 2 g Cr(VI) per vehicle after July 2003 [6, 7]. Therefore, it is urge to found an alternative coating material for Cr(VI). Therefore, in 1951 Cr(III)-based conversion coatings were newly discovered [4, 8]. However, their industrial usage has been put into practice by European directives since restricting hexavalent chromium from being used in surface finishing industries[9, 10]. On comparing the both Trivalent and hexavalent Chromium-based Conversion, the Cr (III) conversion coatings maintains the large scale of resisting temperature up to 150°C while Cr (VI) conversion coatings maintains only 60°C resisting temperature. [4, 11, 12]. Although the Cr (III)-based conversion layer also acts as a barrier[13] without heat treatment, the corrosion protection of Cr (VI) coatings is generally better[14].

Cr (III) octahedral complexes are generally inert to the exchange of water molecules with other ligands. Cr (III) has an excellent stability range, a very slow ligand displacement and substitution reactions, which allow separation, persistence, and/or isolation of thermodynamically unstable Cr (III) species[15].

To prepare Cr(III)-based passivation solutions, apart from a Cr(III) salt and additional transition metal ions to increase the corrosion resistance, another component to form a complex with Cr(III), which is kinetically less inert than  $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$ , is needed. In a hexavalent chromium conversion process, the Cr (VI) ion acts as an oxidizing agent, while in the TCC coating process the role of the oxidation agent is mainly carried out by nitrate [4, 16]. In addition, it is convenient for users to manage the use of work pieces, and establish a foolproof mechanism on electroplated plants. However, the strength of dyeing is based on the different components of the passivation agents, which may cause the users to misjudge uncolored [17].

Now a day, the metal oxide/hydroxide pays more attention in the field of electrochemical studies[18]. This is because of, the unique properties such as intrinsic nature, easily abundant, larger surface area, give more catalytic activity etc [19]. In addition, uric acid is one of the most common biomolecule, which commonly exist in the biological fluids such as urine and blood serum. The excess level of uric acid leads to the various syndrome such as Parkinson's disease and schizophrenia. So that, the trace level detection of uric acid in biological samples is necessary one. There are different types of techniques are available to detect the urine sample such as high performance liquid chromatography (HPLC)[20], liquid chromatography electrospray tandem, and fluorescence-based screening assay etc [21] . However, these techniques are time consuming, high cost, consume more sample[22].

In the past decade, the modified electrode based electrochemical sensor pays more attention in the detection of biological samples. However, it is an important to found suitable electrode materials to detect the biomolecules. Zinc-based oxide and hydroxide materials shows an exceptional electrochemical behaviors and having excellent thermal conductivity[23, 24]. Considerably, the zinc hydroxide coated on iron substrate ( $\text{Zn}(\text{OH})_2$ ) modified electrode is subjected to the detection of UA.

This study aims to enable the electroplating industry achieve the goals of environmental protection, cost reduction and biomolecule detection. Therefore, focus on the major factors that affected

coloring and optimized the trivalent chromium passivation dyeing process. In addition, Zinc hydroxide coated iron substrate was used for the electrochemical detection of uric acid in the urine samples, which shows the excellent wider range and limit of detection.

## 2. EXPERIMENTAL

### 2.1. Materials

The substrate for testing Zinc plated tapping screw (type: M3, size: 50mm) was used as a substrate for testing.  $ZnCl_2$ , Uric acid, NaOH,  $HNO_3$  (70%) and  $H_2SO_4$  (98%) chemicals were purchased from Sigma Aldrich and used as received. CRT-150, BK-25 were used as passivation agents and EVERANOD® Yellow RS 01(K750), EVERANOD® Blue RS 01(K751), EVERANOD® Orange RS 01(K752) were used as dyestuffs for the experiments.

### 2.2. Methods

Cooling water circulation device (0~30°C), model: P-20 Sheng Hsin instruments, thermal water bath tank (30 ~ 99°C), DENG YONG G-20 electronic weight scale (0.1 mg), Precisa XS 625M, UV-Visible Spectrometer: SHIMIDAZU UV-1800, Electro conductivity meter (SUNTEX SC-2300 (0 $\mu$ s / cm ~ 200ms / cm)), 50ml centrifuge tube, Bilfill, Zeta potential meter: Mutek™ SZP- 06, Corning stirrer hot plate PC620, Oven (100 ° C), Haishan CO-05, Multi-light source color light box, GAIN BONA SIGHT were used for the characterizations. The supporting electrolyte consumed for the electrochemical studies is 0.1 M phosphate buffer pH 7. The electrochemical experiments were carried out through CHI 1205B workstation.

### 2.3 Experimental method:

#### 2.3.1 Passivation dyeing process steps:

Work pieces degreasing → Rinsing → Activation → Rinsing → Passivation dyeing → Rinsing → Drying → Color evaluation.

#### 2.3.2. Activation, Passivation dyeing, Dry and color evaluation

(1) Dipping the screw in diluted  $HNO_3$  solution (10ml / L) at room temp. (25°C) for 3 seconds to activate the surface

(2) Moving the screw to rising with RO water

(3) Pre-dissolved dye solution: Take 2g each of EVERANOD K750, K751, and K752. Then dissolve in 100ml RO water, put on a hot plate and stir until all dye was dissolved.

- (4) Put the activated screws in a centrifuge tube containing the dye solution (100ml/L) and passivation agent (100ml/L), and shake it 30sec.
- (5) Moving the screw to rising with RO water
- (6) Put the screws in the oven, set 100 °C for 10 minutes.
- (7) Take out the screws and wait for cooling to room temperature.
- (8) Manual color evaluation in the light booth.

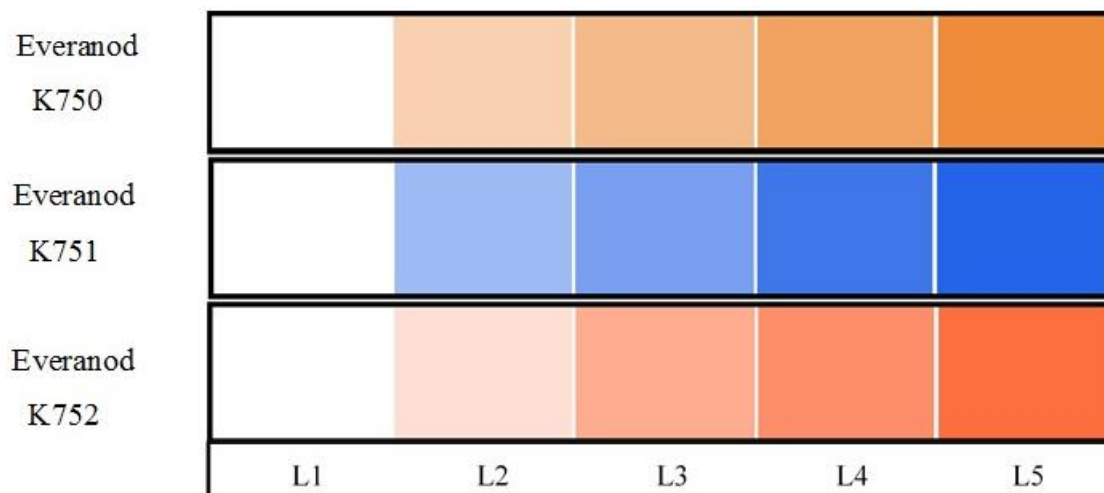
### 2.3.3 Analyze

The color evaluation levels obtained according to different dyeing conditions, and use JMP and Excel statistical software to find out the effect of the coloring factors can effectively improve the coloring strength.

### 2.3.4 Definition of color strength

Since the work piece are screws and the surface is not flat, it cannot be measured by the color-testing instrument. Thus, this experiment uses light booth color evaluation. The following indicators divide the color intensity into 5 levels (level 1: uncolored, level 5: color strength reached saturation)

Applicable dye

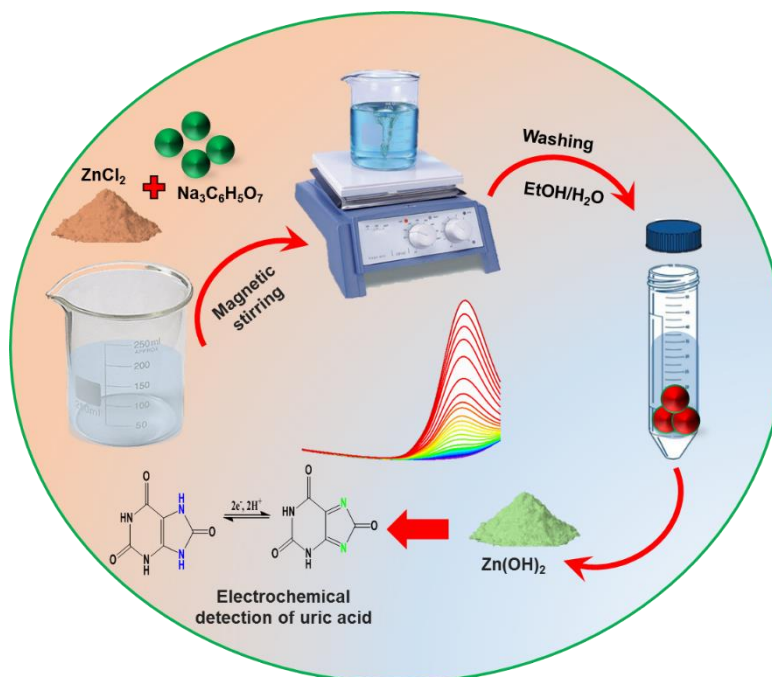


**Figure 1.** Color strength index

### 2.3.5 Synthesis of Zn(OH)<sub>2</sub> nanoparticles

Zn(OH)<sub>2</sub> nanoparticles were prepared through simple co-precipitation method. In typical, 1mmol of ZnCl<sub>2</sub> were prepared in the 5 mL of ethanol. Then the prepared solution was mixed in 50 mL of water, which contain 1 mmol of sodium citrate. Then the prepared solution were allowed to keep in the magnetic

string for 30 min in room temperature. After that, 2 M of 5 mL sodium hydroxide were added drop wise, then the obtain precipitated was centrifuged and washed with water and ethanol for serval times[25]. The prepared  $\text{Zn}(\text{OH})_2$  nanoparticles were deposited on the passivated iron substrate and named as  $\text{Zn}(\text{OH})_2$  NPs modified electrodes. For the control studies passivated iron substrate were used and named to be bare electrodes.



**Scheme 1.** Schematic representation of synthesis methodology of  $\text{Zn}(\text{OH})_2$  NPs and its electrochemical application towards the detection of UA

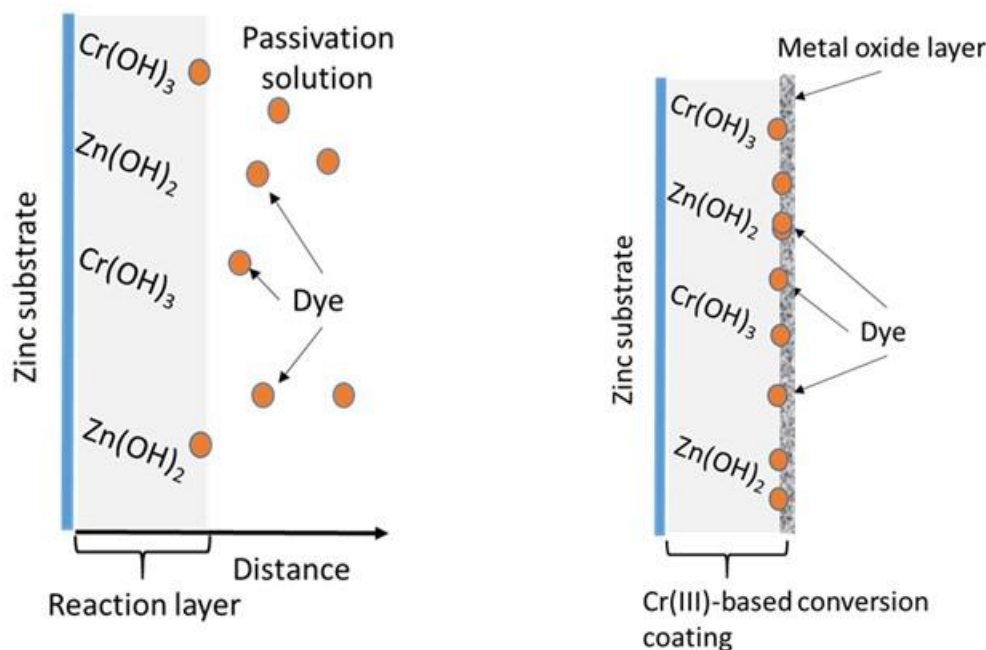
### 3. RESULTS AND DISCUSSION

This study used statistical methods to find the correlation between different parameters, analyze the important factors affecting coloring, and find the best operating conditions. The Zeta potential and electrical conductivity of the solution are used in confirm whether there is a correlation between the strength and the strength to provide a basis for industrial judgment.

#### 3.1 Cr (III) based conversion coating process and dyeing process

Fig .2a depicts the graphical representation of layer produced by Cr (III)-based treatment solution. Fig. 2b explains that, at the time of zinc conversion coating process, a mixture of the dye is move close to the reaction layer between the substrate (zinc) and the bulk electrolyte. The dye may hinder the smooth deposition of metal hydroxide/oxide on the substrate. Throughout the TCC process, as the bubble expands, its internal pressure decreases, and the resultant forces cause the bubble to detach from the reaction layer. After detachment of a bubble, the gas region consists of disjoint volumes and

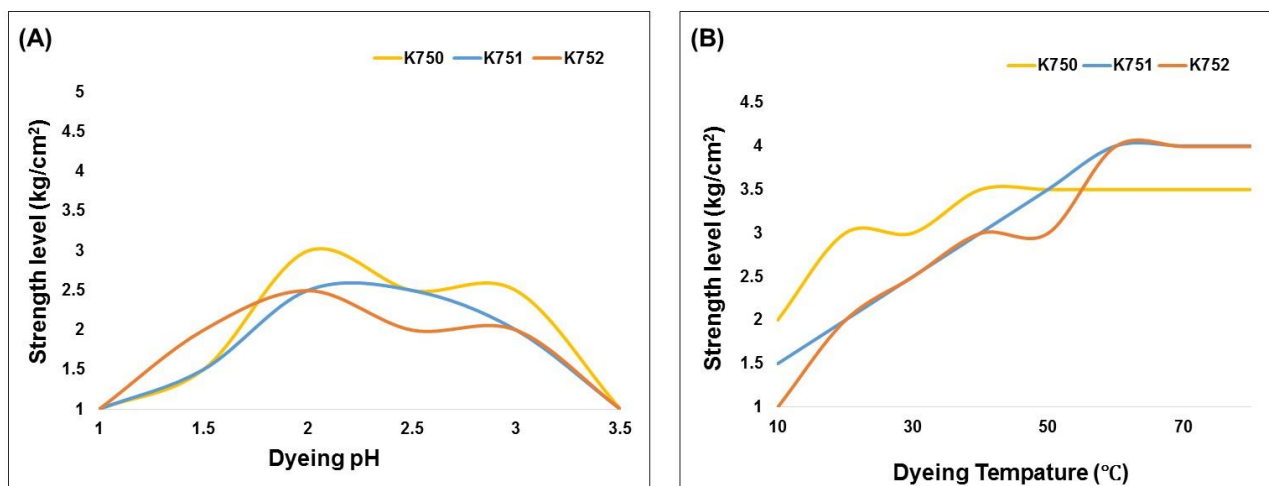
dye can be deposited. However, the TCC process duration might not be long enough to fill the gap with metal hydroxide/oxide depositions.



**Figure 2.** (a) A schematic illustration of forming passivation-coating layer and dyeing (b) A graphical representation of the ultimate morphology of the formed TCC layer after dyeing.

### 3.1.1 Adjusting pH and temperature

This stage of the experiment, three colors (yellow, orange, blue) are used. The test is to adjust the temperature and pH of dyeing for testing. After dyeing, dry and use the color strength index to determine the level. It can be known in Fig. 3A that when the dyeing temperature is 25 °C, the pH is between 2 and 2.5, and the better coloring strength can be obtained. When the pH value is less than 1.5, it is inferred that the zinc layer will dissolve too quickly due to the low pH value, and even damage the plating. If  $\text{pH} > 3.5$ , the zinc layer cannot be colored, because it's difficult to form a passivated porous layer without enough acidic condition, which will also reduce the strength. Refer to the comprehensive evaluation of the strength of the three dyes, so the dyeing pH 2.0 is preferred. In Fig. 3B discusses effect of the dyeing temperature, color strength is saturated and stable at 50 ~ 60 °C. Based on energy consumption and end-user management considerations, the dyeing temperature 60°C is the best choice.



**Figure 3.** (A) Effect of dyeing pH values on strength level. (B) Effect of dyeing temperature on strength level

3.2 statistical verification

Combination of the temperature and pH variations due to verification Table 1:

**Table 1.** Temperature and pH relationship experimental design

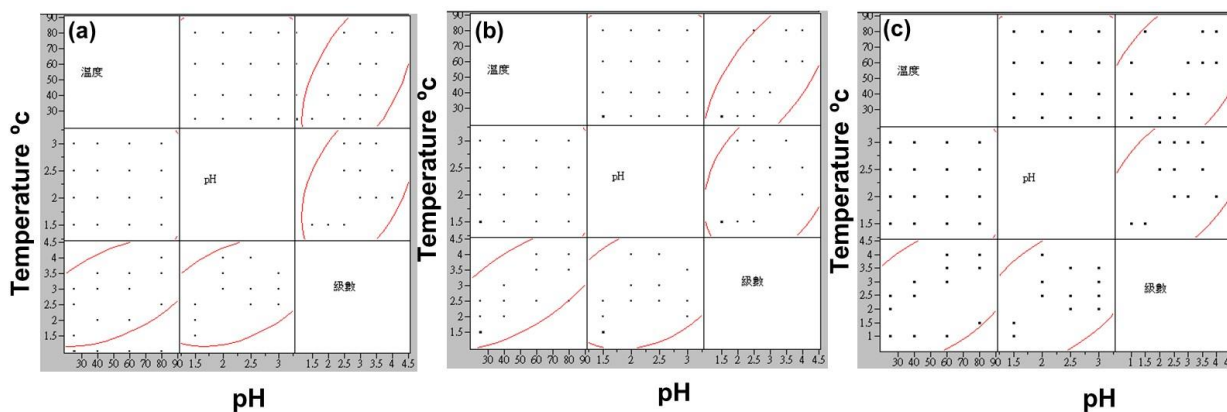
Dyestuffs	Everanod K750, K751, K752
dyeing temperature	25,40,60,80 °C
Dyeing pH pH 2.0	1.5, 2.0, 2.5, 3.0

Based on the data, use JMP software to find the main results that affect coloring. The correlation analysis chart is shown in Table 2. below:

**Table 2.** Both dyeing temperatures and pH values Correlation analysis results

Multivariate	Everanod K750(Yellow)	Everanod K751(Blue)	Everanod K752(Orange)
Dyeing temp.	0.5438( moderate)	0.7030(high)	0.4845( moderate )
Dyeing pH	0.4256( moderate)	0.3192(moderate)	0.4856( moderate )

\*: 0 ~ 0.3: Low correlation 0.3 ~ 0.7: Moderate correlation 0.7 ~ 1.0: High correlation



**Figure 4.** Correlation analysis of different dyeing temperature and pH intensity series at different pH values, (a) Everanod K750 (b) Everanod K751 (c) Everanod K752

It can be known from the correlation analysis that when different dyes have temperature and pH changes at the same time, the changes in strength are positively and moderately correlated, so it is determined that When it is high, it will increase the intensity of coloring, but the temperature is too high and the evapotranspiration needs to be constantly replenished. It is unfavorable for on-site management and energy consumption. It is best to maintain the temperature at 60 ° C. Although the pH value is moderately positively related to the coloring, the main blame is during the passivation dyeing process, when the pH is less than 1.5 and the pH is greater than 3.0, the strength obviously decreases or does not color. This is related to the formation of the passivation layer. This phenomenon causes the correlation to decrease, so pH 2.0 is still the best. Finally, this analysis is also beneficial to the matching of the three dyes, because their correlations belong to the same range, which is conducive to the stable coloring when they are dyed.

### 3.3 Determination of the Zeta potential and conductivity:

Discusses the changes in the Zeta potential and the conductivity of the dyeing solution before and after addition of passivation agents. The passivation agent is CRT-150 and BK-25 witch had different dyeing strength. The following Table 3 and 4 represent the Zeta potential and the conductivity, changes respectively. The results did not show a clear correlation on the coloring. There are other factors affecting the dyeing results of different passivation agents, but it can be inferred that the dye solution is a negatively charged solution. After the passivation solution with a lower pH (about 1.5 ~ 1.8) was added, the charge neutralization was generated, which reduced the Zeta potential, and the passivation solution was rich in electrolyte, which was much larger than the original content of the dyeing solution, resulting in a significant increase in electrical conductivity.



**Table 3.** Effect of adding passivation agents on zeta potential and coloring strength of the dyeing solution

Zeta potential test	K750	K751	K752
dyesolution2g/L, 15ml	-411mV	-757mV	-601mV
After adding 1.5 mL(10%) passivation agents			
CRT-150	-10mV	-7mV	-31mV
BK-25	-9mV	-8mV	-40mV
Strength level(60°C, pH 2.0)			
CRT-150	4	4.5	4
BK-25	2	2	1.5

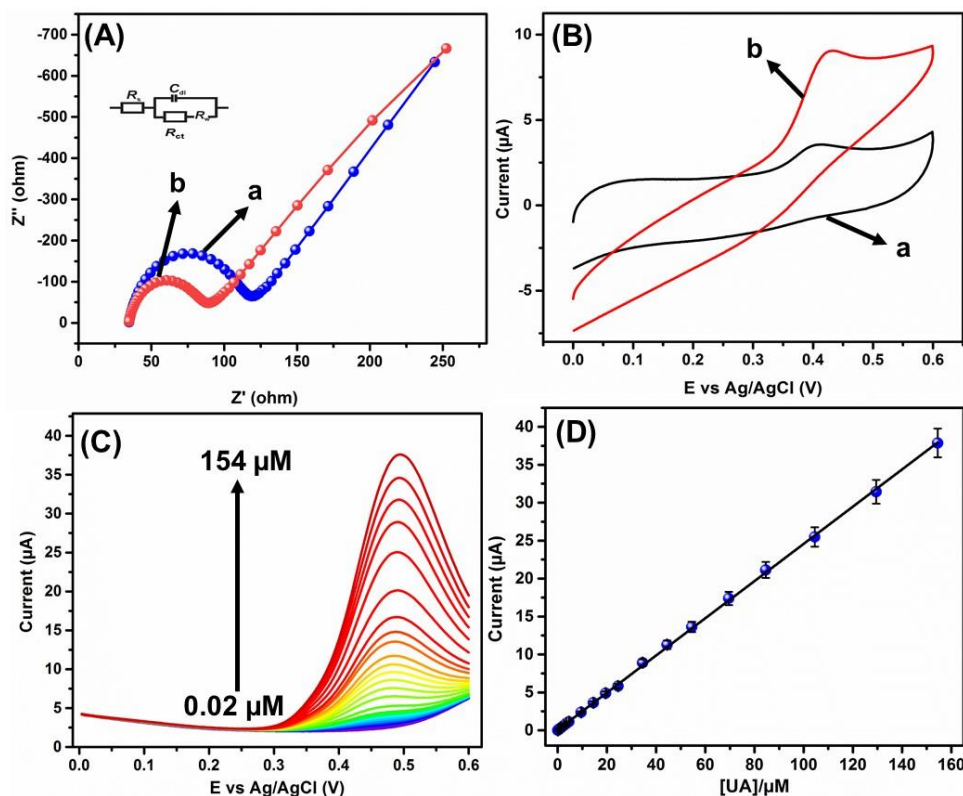
**Table 4.** Effect of adding passivation agents on electrical conductivity and coloring strength of the dyeing solution

EC test	K750	K751	K752
dyesolution2g/L, 15ml	1511*	1229	3540
After adding 10 mL(10%) passivation agents			
CRT-150	35300	34500	34700
BK-25	34500	33800	33900
Strength level(60°C, pH 2.0)			
CRT-150	4	4.5	4
BK-25	2	2	1.5

\*unit:  $\mu\text{s}/\text{cm}$ 

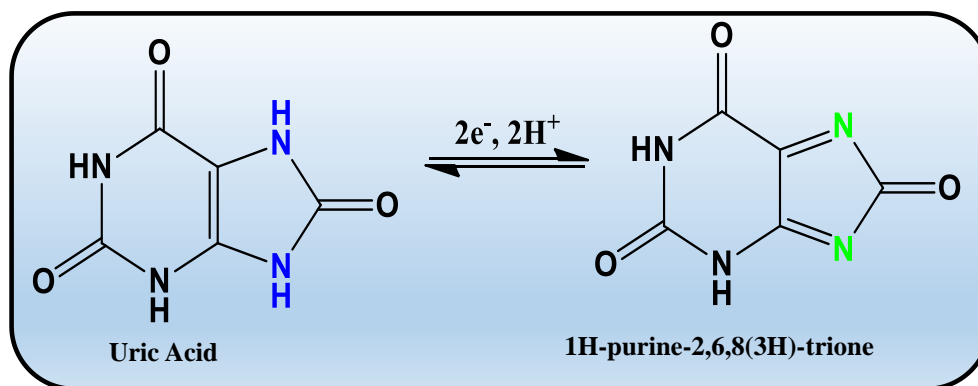
### 3.4. Electrochemical performance

Electrochemical impedance spectroscopy (EIS) is the one of the most common technique, which is used to measure the interfaces between the electrolyte and the surface electrode. Fig. 5A shows the EIS the curve of bare (a) and  $\text{Zn}(\text{OH})_2$  NPs (b) modified electrode in 0.1 M KCl containing 0.05 M of  $[\text{Fe}(\text{CN})_6]^{-3/4}$ . On comparing both the electrode, the lower charge transfer resistance ( $R_{ct}$ ) of 58.24  $\Omega$  was noted on the  $\text{Zn}(\text{OH})_2$  NPs. The  $R_{ct}$  value obtained at bare electrode was measured to be 99.38  $\Omega$  respectively. As the results,  $\text{Zn}(\text{OH})_2$  modified electrode shows the excellent contact between the electrode and electrolyte solution.



**Figure 5.** (A) EIS curve of bare electrode (a) and  $\text{Zn}(\text{OH})_2$  NPs modified electrode (b) in 0.1 M KCl containing 0.05M of  $[\text{Fe}(\text{CN})_6]^{-3/4-}$ . (B) CV's of bare electrode (a) and  $\text{Zn}(\text{OH})_2$  NPs modified electrode contain 50  $\mu\text{M}$  of UA in pH-7. (C) DPV response of UA at  $\text{Zn}(\text{OH})_2$  NPs modified electrode. (D) Linear relation plot of current ( $\mu\text{A}$ ) vs  $[\text{UA}]/\mu\text{M}$ .

Fig. 5B depicts the Cyclic Voltammetry response of (CV's) of bare (a) and  $\text{Zn}(\text{OH})_2$  NPs (b) modified electrode in 0.1 M pH-7 containing 50  $\mu\text{M}$  of uric acid (UA). The lower oxidation peak current of 1.89  $\mu\text{A}$  was noted on bare electrode, this is due to the largest  $R_{ct}$  value. The sharp and well-defined oxidation peak current of 7.82  $\mu\text{A}$  was obtain on  $\text{Zn}(\text{OH})_2$  NPs modified electrode, which suggest the incorporation of  $\text{Zn}(\text{OH})_2$  NPs attribute to increase the sensitivity of the electrode. In addition, the differential pulse voltammetry (DPV) technique was applied to investigate the sensitivity of the material. Fig. 5C shows the DPV response of  $\text{Zn}(\text{OH})_2$  NPs modified electrode in pH-7. On increasing the concentration of UA, the peak current also increased linearly. Fig. 4D depicts the linear relation plot of current versus the concentration of UA. Finally, the electrode shows the linear range of 0.02  $\mu\text{M}$  to 154  $\mu\text{M}$  with the limit of detection (LOD) of 0.204  $\mu\text{M}$ . In addition the linear regression equation is measured to be  $i_{pa}(\mu\text{A}) = 0.02452 \text{ UA } (\mu\text{M}) - 0.0565$ ,  $R^2 = 0.9942$ . The possible electrochemical oxidation mechanism of UA were given in mechanism (1)



**Mechanism 1.** The possible electrochemical oxidation mechanism of UA

### 3.5. Real sample analysis

In addition, the real sample investigation of Zn(OH)<sub>2</sub> NPs modified electrode also done in the DPV technique. For the practicality of the prepared sensor, the real time applicability of UA was investigated in human urine sample. The human urine was collected from the healthier person, and the collected sample were diluted with buffer solution and the known concentration of UA were added by serial dilution method. After that, the DPV response of urine samples were recorded. Finally, the found and recovery value of the modified sensor were calculated and presented in table 5.

**Table 5.** Determination of UA in urine samples at Zn(OH)<sub>2</sub> NPs modified electrode

	Added (nM)	Found (nM)	Recovery (%)	*RSD %
Real Samples				
	250	248.24	99.29	2.54
Human urine	300	291.51	97.17	3.2
	400	386.24	96.56	3.58

## 4. CONCLUSION

In this study, discussed the electrochemical principle on Zinc galvanizing 、 Zeta potential 、 Electrical conductivity 、 IC and ICP OES. In addition, Zn(OH)<sub>2</sub> NPs were prepared through co-precipitation method and passivated on the iron substrate which is used to detect the uric acid in urine sample. Moreover, the modified electrode shows the wider range of 0.02 to 154 μM. The influence of passivation dyeing conditions on the strength of coloring was obtained. Optimized process parameters: Use EVERANOD® Yellow RS 01(K750)、 EVERANOD® Blue RS 01(K751)、 EVERANOD® Orange RS 01(K752) (concentration 20g / L) to take 100ml / L and passivation agent 100ml / L for

mixing and dyeing at a temperature of 60 ° C and pH 2.0 to obtain the best color strength. This condition is helpful for the reference of passivation dyeing in the process of electroplating plants. At the same time, it is also used to determine whether the zeta potential and the conductivity are electrochemical parameters that affect the coloring. Although no effective results have been obtained, it can also try new directions for future research. We hope that the results from the experiment will enhance the advancement of the research and development on more environmentally friendly dyestuffs and biomolecule detection. If there is any new experimental data or related information in this regard, it will be published and shared with all readers in the future.

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