



## Simple Colorimetric Method Using Aqueous Solution to Detect Heavy Metal

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### Abstract

Simple method has been used to determine the absence of heavy metals in an aqueous solution. Fluorescein was used as the base colorimetric material. This was doped with  $\text{CuCl}_2$  and the final solution showed a clear change in color. This change was correlated with the change in both pH and electrical conductivity of the solution. The optical property as an obvious change of the spectra was observed. Therefore, this simple method could be proposed as a method to detect heavy metals in any solution.

**Keywords:** Colorimetric,  $\text{CuCl}_2$  aqueous solution, UV-visible absorption, Conductivity.

### استخدام طريقة لونية بسيطة باستعمال محلول مائي الكشف عن المعادن الثقيلة

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### الخلاصة

تم استخدام طريقة بسيطة لتحديد وجود المعادن الثقيلة في محلول مائي باستخدام صبغة الفلوريسين كمادة أساسية للقياس الألوان، تم استخدام صبغة الفلوريسين مع  $\text{CuCl}_2$  أظهر المحلول النهائي تغيراً واضحاً في اللون بعد إضافة محلول مائي يحتوي على ملح معدن النحاس. يرتبط هذا التغير بتغيير واضح في كل من الرقم الهيدروجيني والموصلية الكهربائية للمحلول والأطياف البصرية حيث لوحظ حدوث تغير واضح في الأطياف. لذلك، يمكن اقتراح الطريقة البسيطة كدالة لتحديد المعدن الثقيل في المحلول.

### 1. Introduction

Heavy metal ions are of great concern among chemists, biologists, and environmentalists; they are very toxic and may cause health and environmental problems, regardless of the significance of some heavy metal ions in living systems [1]. High concentration of heavy metals ions such as Fe(III), Zn(II), Cu(II), Co(II), Mn(II), and Mo(VI) result in negative health effects, however, some of them are essential for the human metabolism [1-3]. Accumulation of such ions in the human bodies over time results in serious debilitating illnesses [4]. Therefore, it is essential to develop new methods to select and determine heavy metal ions. Fluorescein is an organic dye and is useful as a possible searching reagent for latent bloodstains [5]. Developing and synthesizing different fluorescein derivatives are still interesting to meet some requirements in different applications [6, 7]. Several reports that show the basic fluorescence properties of some well-known fluorescein dyes are already published [8-11], however, the experiments was carried out under different conditions and techniques. Therefore, the

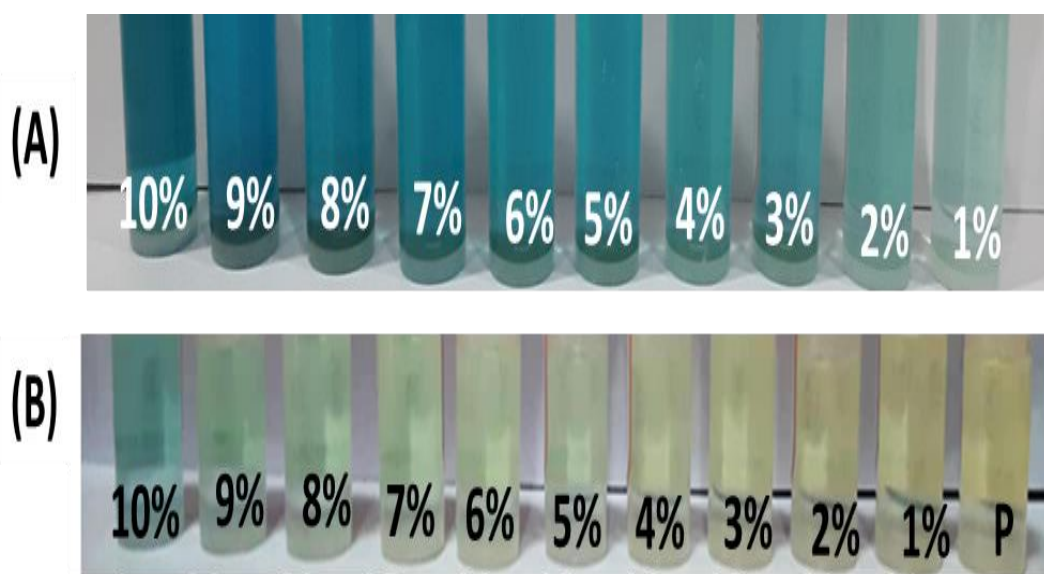
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comparison between the collected data is found to be difficult for different dyes. Fluorescein color is dark orange in the form of powder; however, diluted fluorescein shows bright yellow-green color as a function of the way it is observed: by reflection or by transmission [12]. The ionization equilibrium of fluorescein leads to pH-dependent absorption and emission [13]. Fluorescein complexes might be prepared by binding Cu (II) with the fluorescein molecules; these ligands (copper (II) fluorescein complexes) are sensitive to pH values of the buffered solutions. Lim et al. have studied the use of copper (II) fluorescein complexes to detect nitric oxide (NO) in water [14]. The absorption properties of such complexes have shown binding stoichiometry for the Cu (II) complexes of different fluorescein derivatives in pH 7.0 buffered aqueous solutions. In the current study, the colorimetric detection of the Cu (II) ions in aqueous solution was developed using a simple methods such as pH values, absorption spectra and electrical conductivity. Usually, the Fluorescein dye solution is based on one solvent, in this study a co-solvents of Ethanol: water was used. Different concentrations of  $\text{CuCl}_2$  were used to evaluate the lowest detection ability of such colorimetric sensor.

## 2. Materials and Methods

### 2.1 Samples Preparation

All chemicals and solvents were purchased from Sigma-Aldrich and used without further purification unless otherwise mentioned. Samples were prepared by dissolving the fluorescein dye in 40ml of ethanol to obtain 0.25% mg/ml concentration. On the other hand,  $\text{CuCl}_2$  was dissolved in deionized water to obtain different concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mg/ml). Then, 1ml of ethanol based aqueous luorescein solution (AFS) and 1ml of DI water were mixed to obtain the co-solvents of AFS. After that, 100 $\mu\text{l}$  of water-based  $\text{CuCl}_2$  solution of different concentrations were added to the co-solvents of AFS to obtain the final solutions of AFS:  $\text{CuCl}_2$ . The change in the color is observed upon loading the  $\text{CuCl}_2$  to the AFS as shown in Figure-1.



**Figure 1-**(A) different concentrations of aqueous  $\text{CuCl}_2$  (B) different concentrations of AFS: $\text{CuCl}_2$  where P is AFS

### 2.2. Characterization:

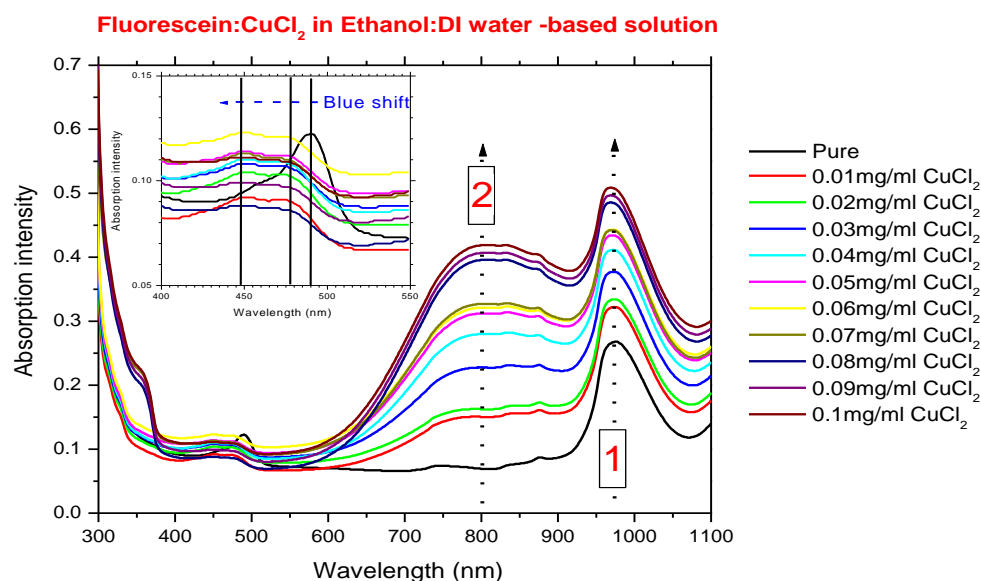
A double beam UV-Visible spectrophotometer (UV-1800 Shimadzu) in the range of 190–1100 nm was used to measure the absorption spectra of aqueous fluorescein solution and aqueous  $\text{CuCl}_2$  solution. The electrical conductivity of the solutions was recorded using DDS307W microprocessor conductivity meter. The pH values of the aqueous solutions were measured using a pH meter (Fisher scientific company).

## 3. Results And Discussion:

### 3.1. UV-Visible Spectrophotometry

Homogeneous mixtures were obtained after mixing co-solvents of AFS and  $\text{CuCl}_2$  solutions, the resulted solution had an obvious change of color upon increasing the  $\text{CuCl}_2$  concentrations as shown in

Figure-1(B). The absorption spectrum of pure fluorescein in ethanol has demonstrated typical behavior with different peaks as shown in Figure-2. The first peak around 490nm is directly related to the fluorescein absorption [15] while the absorption of alcohol solution occurred mainly in the high wavelength regions. The pure fluorescein absorption spectrum has been affected by the addition of  $\text{CuCl}_2$  with different concentrations; low concentrations starting from 10 mg/ml revealed a small change in the absorption spectrum. This has increased with increasing the concentration of  $\text{CuCl}_2$ . The main absorption peak of fluorescein is at around 490nm, this was blue shifted and another peak appeared due to the  $\text{CuCl}_2$  addition. The blue shift was of about 10nm in the main peak and the new peak appeared close to 450nm. The absorption spectra at higher wavelengths demonstrated an increase in the peak intensity with the same position; All the changes were noticed in the range 550-450nm. A shoulder around 380nm was clearly pronounced in the fluorescein based solution which demonstrated a clear blue shift at lower  $\text{CuCl}_2$  concentrations up to 80ng/ml  $\text{CuCl}_2$ . This shoulder has red shifted again to the same position as the pure fluorescein solution. No clear reason was found to explain this behavior and further investigation is needed. The detection limit of this solution for  $\text{Cu}^{+2}$  as a heavy metal in an aqueous solution started from a very low concentration (10ng/ml). The significant color change indicates that this method is a sensitive naked-eye indicator for  $\text{Cu}^{+2}$  detection. Adding  $\text{Cu}^{2+}$  to the fluorescein solution results in a color change from light yellow to light green together with an obvious blue shift in the main absorption band of fluorescein. According to linear Benesi Hildebrand expression, the varied as a function of  $\text{Cu}^{+2}$  ions concentration in a linear relationship, demonstrating the formation of 1:1 stoichiometry between  $\text{Cu}^{+2}$  and fluorescein and this is in good agreement with Li et al.[16]. Typically, the cation-sensing mechanism is based on changing the structure between spirocyclic and open cyclic forms. These probes exist in the spirocyclic forms in the absence of metal ions, which are colorless and non-fluorescent. Adding metal ions results in color change and the generation of strong fluorescence by making the spirocycle open via coordination or irreversible chemical reaction and this in good agreement with Yang et al. [17].

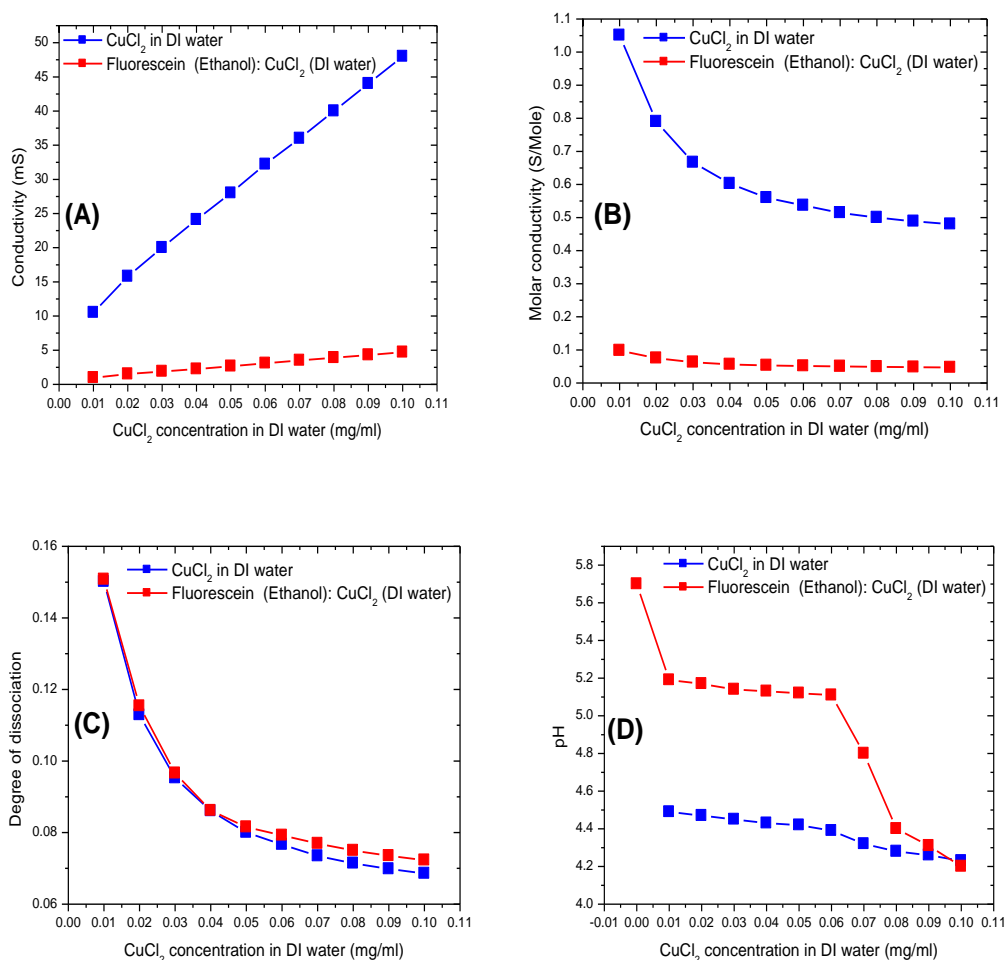


**Figure 2-**UV-Vis absorption spectra of fluorescein:  $\text{CuCl}_2$  solutions with different concentrations

### 3.2 Conductivity

The electrical conductivity increased with increasing the metal salt contents in water; The results are shown in Figure-3. The results demonstrated that the conductivity values increased linearly with increasing the  $\text{CuCl}_2$  concentration in the DI water. This is normally due to the ionic conductivity of the  $\text{Cu}^{+2}$  ions in water, the more content the higher conductivity (see Fig.3A). In an ionic solution like salt water, its conductivity is a function of its concentration and the ability of its charged particles to move freely in the solution. Conductivity of a solution is governed by the number of its charge carriers (the concentrations of the ions) and the mobility of these charge carriers [18]. Theoretically,

conductivity increases in direct proportion to concentration. In practice, this is not accurate, the concentration and mobility of the  $\text{Cu}^{+2}$  ions are not independent properties. As the concentration of an ion increases, its mobility decreases [19].



**Figure 3-**(A) Electrical conductivity, (B) Molar conductivity, (C) Degree of dissociation and (D) pH

On the other hand, adding  $\text{CuCl}_2$  solution to the fluorescein solution increased the conductivity of the fluorescein linearly with values lower than that of the  $\text{CuCl}_2$  aqueous solution. Some ionic compounds are water-soluble, which means they dissolve in water. The molar conductivity ( $\Lambda_m$ ) is defined as the conductivity ( $\sigma$ ) of an electrolyte solution divided by its molar concentration ( $c$ ) as shown in the following equation [18]:

$$\Lambda_m = \sigma/c \quad (1)$$

Once these compounds dissolve, they dissociate, or break into their respective ions. The dissociation of these ions, the degree of dissociation ( $D$ ), is calculated by the following equation [20]:

$$D = \Lambda_m/\Lambda_0 \quad (2)$$

Where  $\Lambda_0$  is the extrapolation of molar conductivity to infinite dilution the limiting value of the molar conductivity. The molar conductivity results are shown in Fig.3B and the degree of dissociation (D) results are shown in Fig.3C. Increasing  $\text{CuCl}_2$  concentration has resulted in decreasing the molar conductivity and the degree of dissociation of the solution. This change is very sensitive to the amount of the metal salts which directly affects the color properties of the solution. At low concentrations, the dissociation of ions is very high resulting in higher molar conductivity. Once the  $\text{Cu}^{+2}$  ions increased in the solution, the dissociation decreased resulting in lowering the mobility of the ions and then decreasing the molar conductivity [19]. This decrease is found to be in good correlation with the pH values of these solutions; pH has demonstrated a decrease with increasing the  $\text{CuCl}_2$  concentration as shown in Figure-3D. Once the  $\text{CuCl}_2$  is dissolved in water, it forms a weak base, this is because the

chloride ions can act as the conjugate base of hydrochloric acid. Because HCl is a strong acid (completely dissociates) the chloride ions are very weak conjugate bases, even when they come from copper chloride (I or II). Therefore,  $\text{Cu}^{2+}$  ions are acting as Lewis acid and  $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$  can further dissociate releasing  $\text{H}^+$  ions and decreasing the pH.

### Conclusion

The colorimetric properties of Fluorescein dye has been investigated before and after doping with  $\text{CuCl}_2$ . The obvious change in the color corresponding to change of the optical properties especially in the main absorption peak of fluorescein (490nm) which was blue shifted by about 10nm after adding  $\text{CuCl}_2$  with the appearance of a new peak close to 450nm. All the changes in the absorption spectra were noticed in the range 550-450nm. Moreover, the change in the color is also correlated with a change in the electrical conductivity and pH value. The conductivity has increased with increasing the  $\text{Cu}^{+2}$  in the solution while the pH has decreased. The  $\text{Cu}^{2+}$  ions acts as Lewis acid and  $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$  can further dissociate releasing  $\text{H}^+$  ions and decreasing the pH.

### References

1. Quang, D.T. and Kim, J.S. **2010**. Fluoro-and chromogenic chemodosimeters for heavy metal ion detection in solution and biospecimens. *Chemical reviews*, **110**(10): 6280-6301.
2. McRae, R., Bagchi, P., Sumalekshmy, S. and Fahrni, C.J. **2009**. In situ imaging of metals in cells and tissues. *Chemical Reviews*, **109**(10): 4780-4827.
3. Lee, J.W., Jung, H.S., Kwon, P.S., Kim, J.W., Bartsch, R.A., Kim, Y., Kim, S.J. and Kim, J.S. **2008**. Chromofluorescent indicator for intracellular  $\text{Zn}^{2+}/\text{Hg}^{2+}$  dynamic exchange. *Organic letters*, **10**(17): 3801-3804.
4. Wennberg, M., Lundh, T., Bergdahl, I. A., Hallmans, G., Jansson, J.-H., Stegmayr, B., Custodio, H., Skerfving, M. and EnViron, S. **2006**. Time trends in burdens of cadmium, lead, and mercury in the population of northern Sweden. *Environmental research*, **100**(3): 330-338.
5. Seashols, S.J., Cross, H.D., Shrader, D.L. and Rief, A. **2013**. A comparison of chemical enhancements for the detection of latent blood. *Journal of forensic sciences*, **58**(1): 130-133.
6. Patil, V.S., Padalkar, V.S., Phatangare, K.R., Gupta, V.D., Umape, P.G. and Sekar, N. **2012**. Synthesis of new ESIPT-fluorescein: photo physics of pH sensitivity and fluorescence. *The Journal of Physical Chemistry A*, **116**(1): 536-545.
7. Egawa, T., Koide, Y., Hanaoka, K., Komatsu, T., Terai, T., Nagano, T. **2011**. Development of a fluorescein analogue, TokyoMagenta, as a novel scaffold for fluorescence probes in red region. *Chem Commun* **47**(14): 4162-4164.
8. Yao, H. and Jockusch, R.A. **2013**. Fluorescence and electronic action spectroscopy of mass-selected gas-phase fluorescein, 2'-7'-dichlorofluorescein, and 2'-7'-difluorofluorescein ions. *The Journal of Physical Chemistry A* **117**(6): 1351-1359.
9. Zhang, X.F., Zhang, I., Liu L, **2010**. Photo physics of halogenated fluoresceins: involvement of both intramolecular electron transfer and heavy atom effect in the deactivation of excited states. *Photo chem Photo boil.* **86**(3):492-498 8.
10. Siejak P. and Fraüickowiak, D. **2005**. Spectral properties of fluorescein molecules in water with the addition of a colloidal suspension of silver. *Journal of Physical Chemistry B*, **109**(30): 14382-14386.
11. Orte, A., Crovetto, L., Talavera, E.M., Boens, N. and Alvarez-Pez, J.M. **2005**. Absorption and emission study of 2', 7'-difluorofluorescein and its excited-state buffer-mediated proton exchange reactions. *The Journal of Physical Chemistry A* **109**(5): 734-737.
12. Duarte, F.J., Kelley, P., Hillman, L.W. and Liao, P.F., **1990**. "Dye laser principles: with applications". Academic Press.
13. Baruah, M., Qin, W., Flors, C., Hofkens, J., Vallee, R.A., Beljonne, D., Van der Auweraer, M., De Borggraeve, W.M. and Boens, N., **2006**. Solvent and pH dependent fluorescent properties of a dimethylaminostyryl borondipyromethene dye in solution". *The Journal of Physical Chemistry A* **110**(18): 5998-6009.
14. Lim, M.H., Wong, B.A., Pitcock, W.H., Mokshagundam, D., Baik, M.H. and Lippard, S.J., **2006**. Direct nitric oxide detection in aqueous solution by copper (II) fluorescein complexes. *Journal of the American Chemical Society*, **128**(44): 14364-14373.

15. Earp, A., Hanson, C.E., Ralph, P.J., Brando, V.E., Allen, S., Baird, M., Clementson, L., Daniel, P., Dekker, A.G., Fearn, P.R. and Parslow, J. **2011**. Review of fluorescent standards for calibration of in situ fluorometers: Recommendations applied in coastal and ocean observing programs. *Optics Express* **19**(27): 26768-26782.
16. Li, T., Yang, Z., Li, Y., Liu, Z., Qi, G. and Wang, B. **2011**. A novel fluorescein derivative as a colorimetric chemosensor for detecting copper (II) ion. *Dyes and Pigments*, **88**(1): 103-108.
17. Yang, XF, Li, Y. and Bai, Q. **2007**. A highly selective and sensitive fluorescein-based chemodosimeter for Hg<sup>2+</sup> ions in aqueous media. *Analytica Chimica Acta* **584**(1): 95-100
18. Abdul-Kareem, J., Al-Bermany, Burak Yahya Kadem, **2012** Study the Effect of Iron (III) Chloride on the Viscosity and Conductivity of Polyvinyl Alcohol as an Aqueous Solution at (295.15 K.), *International Review of PHYSICS*, **6**(1): 63-67.
19. Ferreira Nascimento, M.L. **2013**. Determination of Mobility and Charge Carriers Concentration from Ionic Conductivity in Sodium Germanate Glasses above and below. *ISRN Electrochemistry*, Volume 2013, Article ID 240571, 10 pages.
20. Barthel, J.M., Krienke, H. and Kunz, W. **1998**. Physical chemistry of electrolyte solutions: modern aspects. *Springer Science & Business Media*, (Vol. 5).