Methyl Red: A Fluorescent Sensor for Hg$^{2+}$ over Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$

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Abstract

Methyl red (MR), a very well known acid base indicator ($pK_a = 5.2$), shows fluorescence emission in the range 320 nm to 480 nm, with an emission maximum at 375 nm on excitation by photons at 310 nm. A fluorescent intensity titration of MR by Hg$^{2+}$ ion, a well known toxic heavy metal ion, shows quenching of the fluorescent intensity of MR. The quenching continues till the concentration ratio between MR and Hg$^{2+}$ ion becomes 1:1. Similar titration with a number of other metal ions, namely Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$, has very small effect on the fluorescent intensity of MR. Thus MR acts as a selective fluorescent sensor for Hg$^{2+}$ ion by selective fluorescence quenching. The efficiency of fluorescence quenching value is found to be 8.0. The binding ratio and binding constant ($\beta$) with an emission maximum at 375 nm on excitation by photons at 310 nm. A fluorescent intensity titration of MR by Hg$^{2+}$ ion, a well known toxic heavy metal ion, shows quenching of the fluorescent intensity of MR. The quenching continues till the concentration ratio between MR and Hg$^{2+}$ ion becomes 1:1. Similar titration with a number of other metal ions, namely Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$, has very small effect on the fluorescent intensity of MR. Thus MR acts as a selective fluorescent sensor for Hg$^{2+}$ ion by selective fluorescence quenching. The efficiency of fluorescence quenching value is found to be 8.0. The binding ratio and binding constant ($\beta$) between MR and Hg$^{2+}$ ion are calculated from the fluorescence and UV/visible spectral data, and are found to be 1:1 and log $\beta$ = 4.68. The Hg:MR complex was found to be stable in the pH range 5.5 to 8.2.

Keywords: Fluorescence, Hg$^{2+}$ ion, Methyl Red, Quenching, Sensor

1. Introduction

Mercury (Hg) pollution is a global problem, with both nature and humans contributing to it [1]. The sources of Hg are various geological activities, burning coal, mine tailing, waste from chlorine-alkali industries, etc. [2]. In nature, Hg$^{2+}$ is converted into organomercury of the type CH$_3$HgX where X = Cl$^-$, AcO$^-$, etc. Being lipophilic, organomercury compounds can easily accumulate in the human body [3]. Accumulation of organomercury in the human body causes severe damage to many organs, including the central nervous system (CNS). Brain damage, kidney failure and various cognitive and motion disorders are the major toxic effects of Hg.

A number of techniques have been employed to detect Hg$^{2+}$ ions, such as atomic absorption spectroscopy [4], X-ray fluorescence spectroscopy [5], UV/visible spectroscopy [6], electrochemistry [7, 8], inductively coupled plasma atomic emission spectrometry [9], and inductively coupled plasma mass spectrometry [10]. Fluorescence, as a method for detection of heavy metal ions, including the Hg$^{2+}$ ion, has the advantage of high sensitivity and easy application, compared to other techniques [11, 12].

Fluorescence as a technique to detect Hg$^{2+}$ ion is of current research interest. A rhodamine-cyclen conjugate has been reported as a highly sensitive and selective fluorescent chemosensor for Hg$^{2+}$ ion [13]. The high emission selectivity has been reasoned to be due to the formation of a 1:2 complex between the sensor and Hg$^{2+}$ ion, leading to spirocycle opening of the sensor. 8-hydroxyporphinol derivative having an appended boron-dipyromethene has been reported to detect Hg$^{2+}$ ion by fluorescent “on-off” mode [14]. A new probe, 1,4-bis(1-pyrenyl)-2,3-diaza-1,3-butadiene, was reported for selective sensing of Hg$^{2+}$ ion through a yellow to deep-pink color change, with an enhancement of the fluorescence accompanied by red shift of the excimer emission [15]. Cyclams have been derivatized with two different fluorophores-pyrene and nitrobenzoazide (NBD) which were found to detect Hg$^{2+}$ by fluorescence quenching [16]. There are other reports of cyclams as fluorescent sensors for Hg$^{2+}$ ion [17, 18]. Crown ether diazatetraethene, when modified by bis(pyrene) derivative, acted as a fluorescent sensor for Hg$^{2+}$ ion [19]; and calixarenes have also been studied in this regard [20]. New fluorescent sensors for Hg$^{2+}$ are still attracting the interest of scientists [21, 22]. Most of these methods require cumbersome organic synthesis and high cost of starting materials. Therefore, a sensor for Hg$^{2+}$ ion with an already existing dye molecule should be of importance.

Hg$^{2+}$ is a heavy metal ion, having an electronic configuration of 5d$^10$6s$^2$, and oxygen atoms or nitrogen atoms are good binding sites for it [23]. Methyl red (MR, Fig. 1) is a well known acid-base indicator used in a number of titrimetric estimations. The pKa of MR is 5.2 and it is red below pH 4.4 (cationic form), yellow above pH 6.2 (anionic form), and orange in between (mixture of cationic and anionic). Since the color change of MR into the yellow form ceases at pH 6.2, at pH 7.0 MR should be exclusively in the
anionic form, and interaction with an Hg²⁺ ion should be convenient. Most of the reported sensors mentioned in the previous paragraph have one similarity: the presence of O and N in close proximity. MR also has O and N=N in a close position, hence should act as fluorescent sensor for Hg²⁺ ion.

Although MR is a well known acid base indicator that has been used for a long time, its fluorescent properties have not been investigated. As a well known reagent, if it can be used without any modification to detect a pollutant in aqueous medium at pH ca. 7.0, it should have wide applicability. MR (in 1:1 CH₃OH:H₂O, pH 7.0) has been an answer in this regard, reported in this paper, which is found to be fluorescent active when excited by photons of 310 nm with an emission maximum at 375 nm. Hg²⁺ ion has been found to quench the fluorescent intensity of MR selectively over a number of other metal ions, such as Na⁺, K⁺, Ca²⁺, Mg²⁺, Zn²⁺, and Cd²⁺. The binding constant and stoichiometry of binding obtained from both fluorescence and UV/visible spectroscopic data have also been reported.

2. Materials and Methods

MR was purchased from Merck (Whitehouse Station, NJ, USA), and metal salts (Na₂SO₄, K₂SO₄, CaSO₄, MgSO₄, ZnSO₄, and CdSO₄) were purchased from LOBA Chemie (Colaba, Mumbai, India). The metal salts were recrystallised from hot water before use. The water used was doubly distilled, using a quartz distillation plant. Fluorescent spectra were recorded in a Hitachi 2500 fluorescence spectrophotometer (Tokyo, Japan), using a quartz cuvette. UV/visible spectra were recorded in a Shimadzu UV1800 spectrophotometer (Kyoto, Japan). The pH was measured using a Merck digital pH meter, calibrated using standard buffer solutions prepared from pH tablets (Merck). All the titrations were carried out in a universal buffer. The universal buffer was prepared by mixing 19.5 mL 0.2 M boric acid, 19.5 mL 0.05 M citric acid, and 0.5 mL 0.1 M NaH₂PO₄.

One mM solutions of the metal salts were prepared for the fluorometric and UV/visible spectrophotometric titrations. For fluorometric titrations, 2.0 mL of the MR solution (1.2 × 10⁻⁴ M) was pipetted in a quartz cuvette. Fluorescence spectra were recorded by adding 10 µL of a metal salt solution, using a micropipette. After addition of the metal ion solution, the cuvette was shaken very gently, and allowed to stand for 10 min, before spectra were recorded. Similar experimental procedures were adopted for the UV/visible titrations.

For the I₀/I experiments, a stock solution of 10⁻⁴ M MR was prepared in 1:1 (v/v) CH₃OH:H₂O at pH 7.0. The fluorescence intensity (I₀) was recorded by taking 2 mL of the solution in a quartz cuvette. A metal salt was added to the cuvette so that its concentration was 1.5 × 10⁻⁴ M. It was shaken gently and allowed to stand for 10 min, and the fluorescence intensity (I) was recorded.

3. Results and Discussion

The MR was found to show fluorescent emission in the range 330 to 450 nm with an intensity maximum peak at 375 nm on excitation by 310 nm photons in 1:1 (v/v) CH₃OH:H₂O (universal buffer, pH 7.0). Fig. 2 shows the fluorescent intensity of MR (1.2 × 10⁻⁴ M) at pH 7.0 in 1:1 (v/v) CH₃OH:H₂O. The concentration of Hg²⁺ ion was varied from 0.05 × 10⁻⁴ M to 1.7 × 10⁻⁴ M. Fig. 3 is the plot of the fluorescent intensity against Hg²⁺ ion concentration, and it is clear from the figure that Hg²⁺ ion quenched the fluorescent intensity of MR, until its concentration became 1.2 × 10⁻⁴ M. Hence the concentration ratio [MR]:[Hg²⁺] was 1:1 at saturation when fluorescent quenching ceased. This supports a 1:1 complexation between MR and the Hg²⁺ ion. The quenching efficiency is defined as I₀/I, where I₀ and I are the fluorescence intensity of the sensor molecule in absence and in presence of the analyte respectively [19]. In this present work the quenching efficiency has been calculated to be 8.0.

The effects of a number of other metal ions, namely Na⁺, K⁺, Ca²⁺, Mg²⁺, Zn²⁺, and Cd²⁺, on the fluorescent intensity of MR were investigated. The I₀/I values have been compared in Fig. 4 by a bar diagram. Zn²⁺ ion was found to enhance the fluorescent intensity of MR, while the fluorescent intensity was found to re-
main unaltered for K\(^+\) and Mg\(^{2+}\) ions. A little quenching effect was observed for Ca\(^{2+}\) and Na\(^+\) ions. Hg\(^{2+}\) had shown a quenching of about eight times that of the initial one.

The binding constant and stoichiometry of binding between MR and Hg\(^{2+}\) was calculated as reported \[11\] from the plot of log[(I-I\(_o\))/(I\(_{max}\)-I)] versus log[Hg\(^{2+}\)] (Fig. 5). Here, I\(_o\) is the fluorescence intensity of MR solution when no Hg\(^{2+}\) ion was present, I is the fluorescence intensity of MR solution at a particular concentration of Hg\(^{2+}\) ion, and I\(_{max}\) is the fluorescence intensity of MR solution at a saturated concentration of Hg\(^{2+}\) ion. A least squares fitting of data resulted in a straight line (R\(^2\) = 0.989) with a slope of 1.03, indicating 1:1 binding between MR and Hg\(^{2+}\). The binding constant β was found to be log β = 4.68.

The binding stoichiometry between MR and Hg\(^{2+}\) and the binding constant obtained from fluorescence data were further confirmed by UV/visible spectroscopy results. The UV/visible spectra of MR showed two λ\(_{max}\) values at 270 and 428 nm in 1:1 (v/v) CH\(_3\)OH:H\(_2\)O. Addition of Hg\(^{2+}\) ions gradually made the intensity of 428 nm peak decrease, while the 270 nm peak became a shoulder (Fig. 6) with an isosbestic point at 280 nm. The plot of log[(A\(_{o}\)-A\(_{s}\))/(A\(_{s}\)-A\(_{α}\))] versus log[Hg\(^{2+}\)] was linear. A least squares fitting of the data (R\(^2\) = 0.923) showed that the slope is 1.20, indicating that one Hg\(^{2+}\) ion binds to MR. From the intercept, the log β value was found to be 4.83. These results are in good agreement with those obtained from fluorescence data.

The Hg\(^{2+}\) ion is well known for its fluorescence quenching effect on a fluorophore \[19\]. The quenching effect observed in this work may be explained as reported earlier \[24\]. Hg\(^{2+}\) ion binding might change the relative positions of energetically close-lying ligand-centered \(\pi\pi^*\) and \(n\pi^*\) states, leading to switch ‘off’ possible quenching interaction by the \(n\pi^*\) states.

We studied the effect of pH on the stability of the Hg:MR complex, by recording fluorescence intensity at different pH. For this purpose, 2 mL of a 10\(^{-4}\) M solution of MR in 1:1 (v/v) CH\(_3\)OH:H\(_2\)O was taken in quartz cuvette. The fluorescence intensity was recorded at pH 7.0, and a sufficient amount of solid Hg\(^{2+}\) ion was added in order to get minimum fluorescent intensity (maximum quenched effect). The pH was then changed in the range 3.0 to 10.0, using a pocket pH meter, and the fluorescence was recorded. Concentrated NaOH and HNO\(_3\) solutions were used to minimise the dilution effect. The fluorescence intensity was found to remain in the quenched state between pH 5.5 to 8.2, and precipitation occurred at pH 8.2 and above, due to the formation of Hg(OH)\(_2\). Hence fluorescence could not be measured above pH 8.2. The fluorescent intensity started appearing at pH 5.5, and was found to increase as the pH was further lowered. The color of the solution became typical red for MR at low pH, indicating free MR in the solution. Hence, the Hg:MR complex is stable in the pH range 5.5 to 8.2, and a pH of 7.0 is suitable for performing the titrimetric experiments.

4. Conclusions

We have shown that the well known acid-base indicator MR acts as a fluorescent sensor for Hg\(^{2+}\) ion in 1:1 CH\(_3\)OH:H\(_2\)O (pH 7.0) over the metal ions Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Zn\(^{2+}\), and Cd\(^{2+}\). The fluorescence intensity of MR in 1:1 CH\(_3\)OH:H\(_2\)O is quenched ca. 85% of the original intensity selectively by the Hg\(^{2+}\) ion. Calculation based on fluorescence and UV/visible spectrocopic data showed that one Hg\(^{2+}\) ion binds to one MR, with log β = 4.83, where β is the binding constant. Measurement of fluorescence

Fig. 4. Bar diagram comparing the effect of different metal ions on the I/I\(_o\) value of methyl red (MR). I\(_o\) and I are the fluorescence intensity of MR (10\(^{-4}\) M) in the absence and presence of 1.5 \times 10\(^{-4}\) M metal ions, respectively. Spectra recorded in 1:1 (v/v) CH\(_3\)OH:H\(_2\)O (pH 7.0, universal buffer).

Fig. 5. Plot of log[((I-I\(_o\))/(I-I\(_{max}\))) versus log[Hg\(^{2+}\)] for the titration of methyl red by Hg\(^{2+}\) ion in 1:1 (v/v) CH\(_3\)OH:H\(_2\)O (pH 7.0, universal buffer).

Fig. 6. Effect of Hg\(^{2+}\) ion on the UV/visible spectra of methyl red in 1:1 (v/v) CH\(_3\)OH:H\(_2\)O (pH 7.0, universal buffer).
intensity versus pH showed that the Hg-MR complex was stable in the pH range 5.5 to 8.2. The main advantage of this work over the other reported ones is that to detect Hg\textsuperscript{2+} ion at pH 7.0, there is no requirement for any new synthesis or synthetic modification of a well known molecule.

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**References**