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Detection of Amisulpride Using a Chromium-Salophen Optical Probe

كشف الأميسولبريد باستخدام المجس الضوئي كروم-سالوفين

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Abstract

Patients with psychosis around the world are routinely prescribed anti-psychotic medications. Amisulpride (AMI) is one such medication. In order to detect cases like drug overdose, drug abuse, or intentional poisoning, simple, selective and sensitive probes are required. In the present work, we have synthesized a fluorescent probe (CRSA) for detecting Amisulpride (AMI) and characterized it by FT-IR, NMR and mass spectroscopy. The probe emits green light in ethanol and acts as a "turn-off" luminescent sensor for the anti-psychotic drug Amisulpride (AMI) with a quenching percentage of 94% and an excellent limit of detection of 1.6 µM. The sensor responded effectively to only AMI even in the presence of other drugs like sertraline, fluoxetine, escitalopram, paroxetine, olanzapine and clozapine ensuring good selectivity and specificity of the method. The effect of pH on the sensing abilities CRSA and the applicability of the method to real-life samples were also studied using spiked alcohol samples.

Keywords: Forensic sciences, Amisulpride, Schiff base, drug detection, fluorescence analysis method, drug overdose.

المستخلص

يتم إعطاء مرضى الفصام في جميع أنحاء العالم بشكل روتيني أدوية مضادة للذهان. من أجل اكتشاف حالات مثل: جرعة زائدة من المخدرات أو تعاطي المخدرات، أو التسمم المتعمد، وهناك حاجة إلى مجسات بسيطة وانتقائية وحساسة. في هذا العمل، قمنا بتصنيع مجس فلورسنتي (CRSA) وتمييزه بواسطة FT-IR و NMR وقياس الطيف الكتلي. يصدر المجس ضوءًا أخضر في الإيثان ويعمل كمستشعر إطفاء للضوء للعقار المضاد للذهان أميسولبريد (AMI) بنسبة إطفاء تبلغ 94 ٪ وحدًا ممتازًا للكشف يبلغ 1.6 ميكرومتر. استجاب المستشعر بشكل فعال فقط لـ AMI حتى في وجود أدوية أخرى مثل: سيرترالين، وفلوكستين، وإسكيتالوبرام، وبروكسيتين، وأولانزابين، وكلوزابين، مما يضمن تحديدًا وانتقائية جيدة لهذه الطريقة. كما تمت دراسة تأثير الأس الهيدروجيني على قدرات الاستشعار لـ CRSA وإمكانية تطبيق الطريقة على عينات واقعية باستخدام عينات كحولية تم إضافتها لها.

الكلمات المفتاحية: علوم الأدلة الجنائية، أميسولبريد، قاعدة شيف، كشف المخدرات، أسلوب تحليل الفلورسنت، جرعة زائدة من المخدرات.

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1. Introduction

For medical conditions like schizophrenia and other psychiatric disorders; often a combination of drugs are prescribed to alleviate the varied symptoms of the disease [1]. In very rare cases it has been observed that atypical antipsychotic medications like clozapine, olanzapine, can cause fatality at supratherapeutic or even at therapeutic doses [2].

An example of an atypical antipsychotic drug belonging to the class of benzamides is Amisulpride (AMI). Pharmacologically, it is a dopamine receptor antagonist and is used to treat schizophrenia [3]. In very low doses it can be used as an antiemetic to reduce post-operative nausea and vomiting [4,5]. Therapeutically, it also has anti-depressant properties and is used in treatment of dysthymia in different doses [6]. However, an overdose of amisulpride can cause severe cardiotoxicity leading to fatality. Anti-muscarinic delirium and neuroleptic malignant syndrome (NMS) are two more clinical overdose disorders [7]. Additionally, there have been occurrences of intentional poisoning also using AMI [8-12].

During a drug overdose or poisoning investigation, a forensic toxicologist is tasked with identifying drugs and other toxins from tissues, body fluids and other non-biological matrices. A handy aid to narrow down the criminal investigation is the use of simple sensors and optical probes that require minimal sample preparation and can qualitatively identify a molecule from a complex mixture of biomolecules and other toxins.

In the present work, we report the synthesis of a chromium-salophen fluorescent complex (CRSA) which is efficiently used as a sensor for the identification of AMI from a mixture of various drugs. So far all reported methods for the qualitative and quantitative identification of AMI required sophisticated analytical techniques, for extraction and identification like chromatographic, solid phase extraction and electrochemistry [13-19].

Salophens and metallosalophens are a class of chelate ligands studied extensively for their applications in the field of catalysis, medicinal chemistry and sensors. [20-23]. Chromium-Salophen complexes have been used extensively as catalyst for oxidation reactions [24]; but the bright green fluorescence of the complex piqued our interest and its sensing abilities with various antipsychotic drugs were studied. It was observed that it acted as a highly efficient “turn-off” sensor for AMI in a presence of various other drugs. The “turn-off” mechanism was found to be effective only under acidic to neutral conditions and the sensor could efficiently detect AMI from spiked samples of various alcoholic drinks.

The method reported here will facilitate the identification of AMI in forensic toxicology because of the ease of detection without any sophisticated instrumental methods, the simple synthesis of the CRSA probe, its selectivity and low detection limits towards AMI and the real-world application of the method

2. Materials and Methods

All reagents were purchased from commercial suppliers (HPLC Lab, Lobachemie and SRL) and were used without purification.

The UV-vis spectra were recorded on a Shimadzu 1800 Japan UV-visible spectrophotometer. FT-IR ATR spectra were recorded using an Alpha-Bruker

Fourier transform Infrared Spectrometer. The fluorescence spectra were recorded on fluoromax-4 spectrofluorometer (Horiba Japan). 1H-NMR and 13C-NMR spectra were acquired using a Bruker Avance Neo NMR Spectrometer (400 MHz for 1H), using DMSO as a solvent and tetramethyl silane (TMS) as reference. The mass spectra were acquired using the SCIEX QTRAP4500 ESI Mass spectrometer.

All solutions for the studies were prepared using analytical grade methanol. Amisulpride (Sulpitac-200), Sertraline (Serta-100), Fluoxetine (Flunil-60), Escitalopram (C-Pram S-10), Paroxetine (Paxidep CR-25), Olanzapine (Oleanz- 100) and Clozapine (Sizpine- 25 mg) were all obtained as gift samples from a local pharmacy.

2.1. Synthesis of Salophen Ligand (SALP):

The synthesis of the SALP and CRSA was based on previously reported method with some modifications [25] as shown in Figure 1.

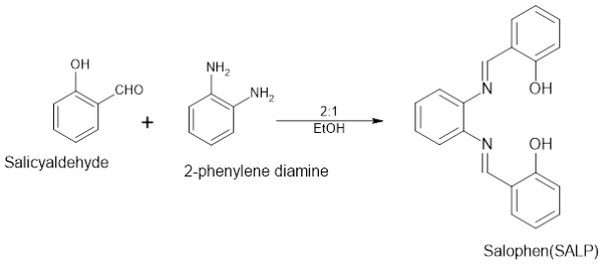

Figure 1- *Synthesis of Salophen*

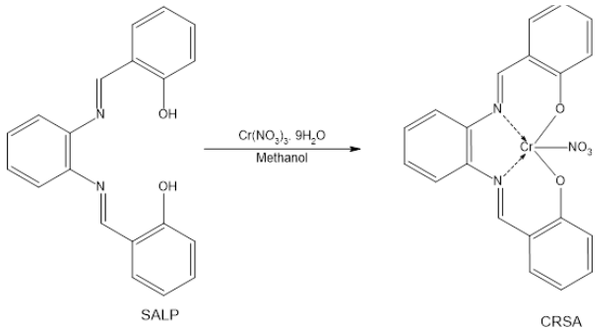

Figure 2- *Synthesis of CRSA*

A solution of salicylaldehyde (0.8 mL, 8.0 mmol) in 5 ml ethanol added slowly to a solution of 2-phenylenediamine (0.43 g, 4.0 mmol) in 5 ml ethanol. The reaction was refluxed for 6 h wherein a yellow-orange crystalline solid was obtained. The Schiff base ligand was filtered, washed with cold ethanol and then air dried to obtain yellow-orange needle-like crystals (2.3 g, 90%).

1H NMR (400 MHz, DMSO-d6): δ 13.00 (s, 2H), 8.92 (s, 2H), 7.67–7.65 (2H), 7.46–7.38 (6H), 6.99-6.95 (t, 4H); 13C NMR (100 MHz, DMSO): δ 164.49, 160.89, 142.72, 133.89, 132.95, 128.27, 120.19, 119.94, 119.54, 117.15, 117.49; IR (cm–1): 3441, 1686, 1611, 1561, 1479, 1275, 1190; ESI-MS: m/z = 316.13

2.2. Synthesis of Cr (III) Salophen (CRSA)

A solution of Cr(NO3)3.9H2O (1.60 g, 4.0 mmol) in methanol (2.0 mL) was added dropwise to a solution of SALP (1.26 g, 4.0 mmol) in methanol (8.0 mL) with stirring. The mixture was then maintained at room temperature for 6 h. The solvent was removed under reduced pressure. The precipitated complex SALP-Cr (III) was then filtered and washed with ethanol. The solid obtained is green in colour (1.43 g, 84%) IR (KBr, cm– 1): 1610, 1573, 1466, 1249, 1198, 1178, 1097, ESI-MS: m/z = 428.32.

2.3. Fluorescence Experiments

A stock solution of 1 x 10-5 M was prepared by dissolving 0.021 g of CRSA in 500 ml ethanol for fluorimetric studies. Stock solution of AMI was prepared dissolving 200 mg tablet in 10ml ethanol and by further diluting it to get a final concentration of 2.7 x 10-4 M. Similarly, stock solutions of Paroxe-

tine, Escitalopram, Fluoxetine, Sertraline, Olanzapine and Clozapine were also prepared in ethanol.

For the detection studies, 1.5 ml (1 x 10-5 M) of CRSA was added to 1.5 ml (2.7 x 10-4 M) of the different anti-psychotic drugs. For the selectivity studies, 1.5 ml of AMI was added to a mixture of CRSA and other drugs. To determine the relationship between fluorescence intensity and concentration of AMI, different concentrations of AMI was added into the CRSA (1.5 ml).

To investigate the effect of pH on the sensing properties of CRSA; 1.4 ml of CRSA and 1.4 ml of AMI were mixed with 2.0 ml of buffer solutions of pH 2.6, 3.1, 4.6, 7.4, 9.2, 9.9 and 10.6.

2.4. Sample Preparation-Drink Spiking

The following alcoholic drinks were spiked with the drug i.e., Old Monk rum, Blender's Pride whiskey, Smirnoff Vodka, Fratelli Cabernet Franc Shiraz wine. Each drink was spiked with different drug solutions to give a final concentration of 0.2 mg/ml AMI. The samples were further used for analysis without any sample treatment or extraction.

3. Results & Discussion

3.1 Characterization of SALP and CRSA

The typical absorption bands for SALP were found at 3441 cm–1, 1686 cm–1, 1611 cm–1, which correspond to -OH stretching vibration, C=N (imine vibration), and O-H bending vibrations respectively. The CRSA complex spectra displayed distinctive absorption bands at 1610 cm–1, 1573

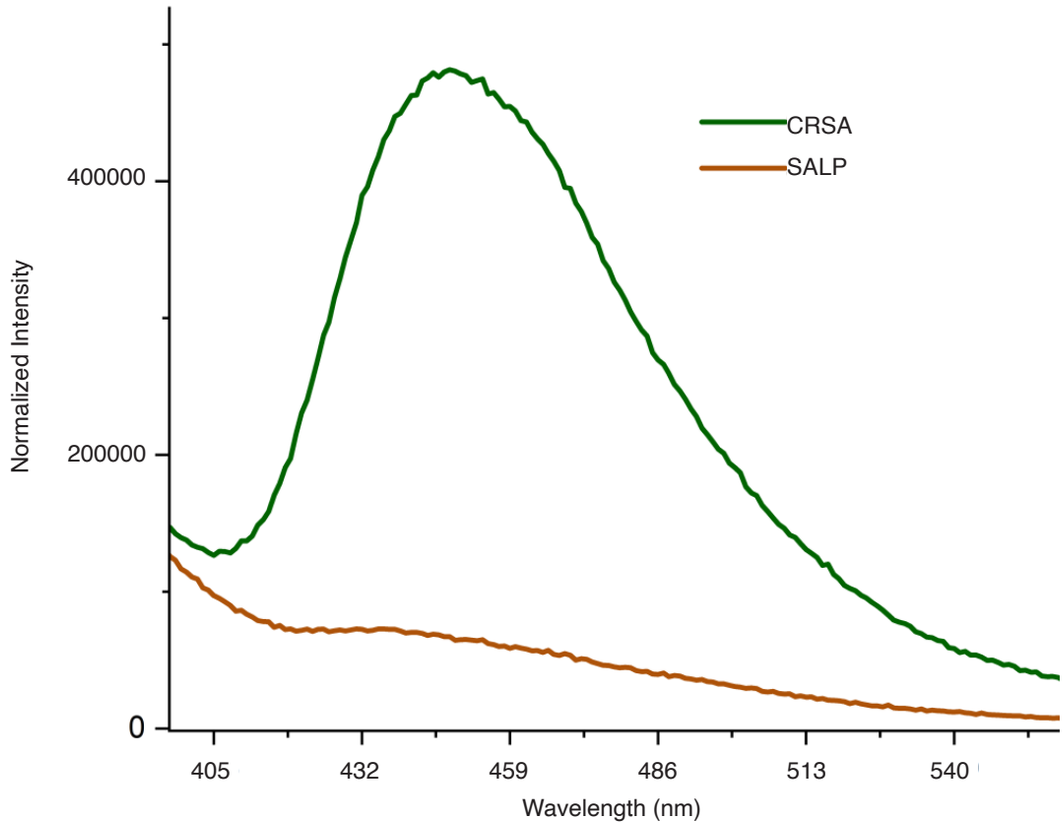

Figure 3- *Fluorescence studies of SALP and CRSA*

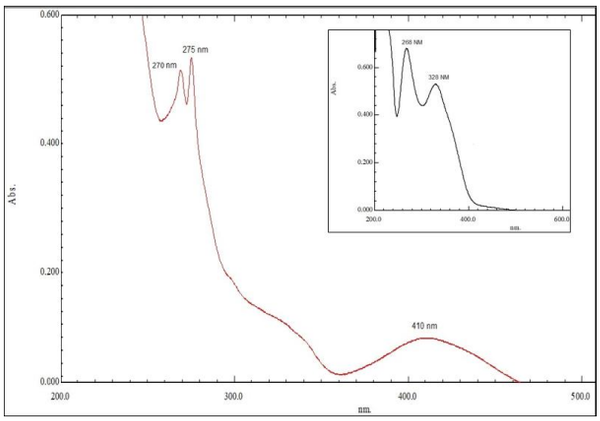

Figure 4- *UV-Vis Spectrum of CRSA (inset UV-Vis spectrum of SALP)*

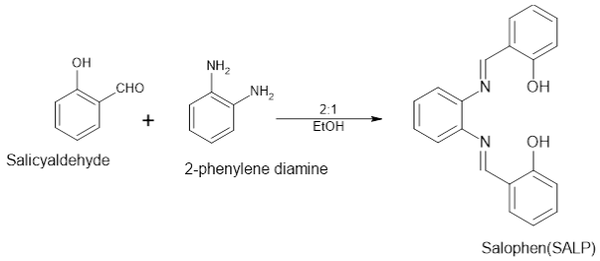

Figure 5- *Emission response of CRSA towards (1) Paroxetine (2) Escitalopram (3)Fluoxetine) (4) Sertraline (5) Olanzapine (6) Clozapine (7) AMI*

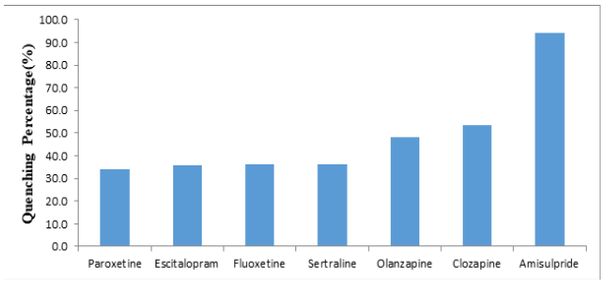

Figure 6- *Quenching percentage of various drugs with CRSA*

cm–1 which are slightly shifted from SALP indicating complexation and absence of the –OH pointing towards its involvement in the complexation of chromium. The mass spectrum also confirms the complexation of chromium by SALP. The fluorescence of SALP and CRSA are analyzed in fluorimeter for their comparative intensity of fluorescence. The non-fluorescent SALP becomes fluorescent

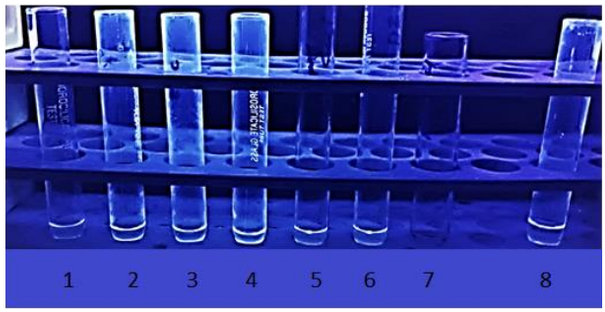

Figure 7- *CRSA (8) with (1) Paroxetine (2) Escitalopram (3) Fluoxetine (4) Sertraline (5) Olanzapine (6) Clozapine (7) AMI under UV lamp*

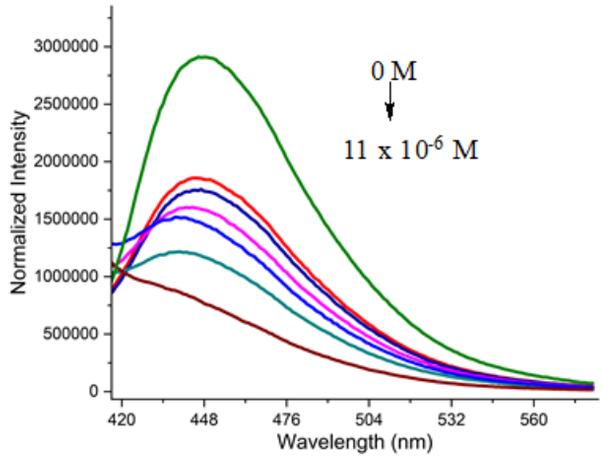

Figure 8- *Variation in emission intensity of AMI upon gradually decreasing concentration of AMI*

when combined with chromium ion as shown in Figure 3 [26].

The UV-Vis spectra of SALP and CRSA are as shown in Figure 4. The absorption spectra of CRSA exhibits peaks at 410, 330, 275 and 270 which corresponds to charge transfer, n $\longrightarrow$ $\pi^*$and $\pi \longrightarrow \pi^*$ transitions.

3.2. Selective Sensing of Amisulpride

Various anti-depressants and anti-psychotics in ethanol were studied to investigate the usefulness of CRSA as a sensor Figure 5. The emission at 444 nm is very minimally disturbed due to presence of paroxetine, escitalopram, fluoxetine, sertraline,

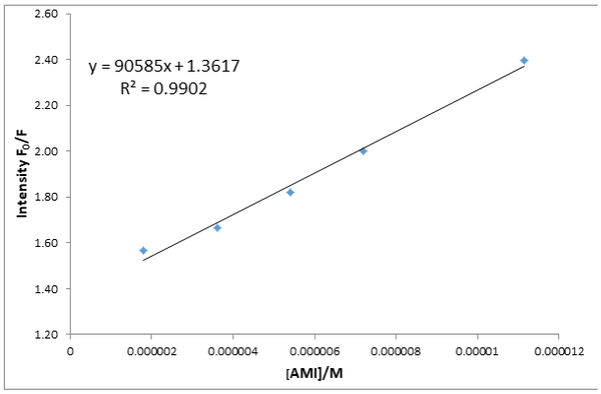

Figure 9- *Stern Volmer plot of quenching of CRSA by Amisulpride*

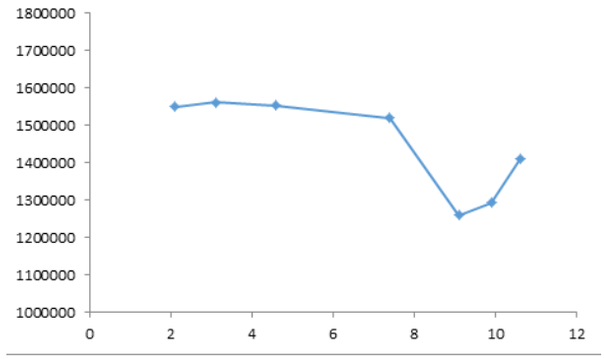

Figure 10- *pH studies on AMI x CRSA reaction. Acidic pH-2.6, 3.1, 4.6. Neutral pH- 7.4. Alkaline pH-9.2, 9.9, 10.6*

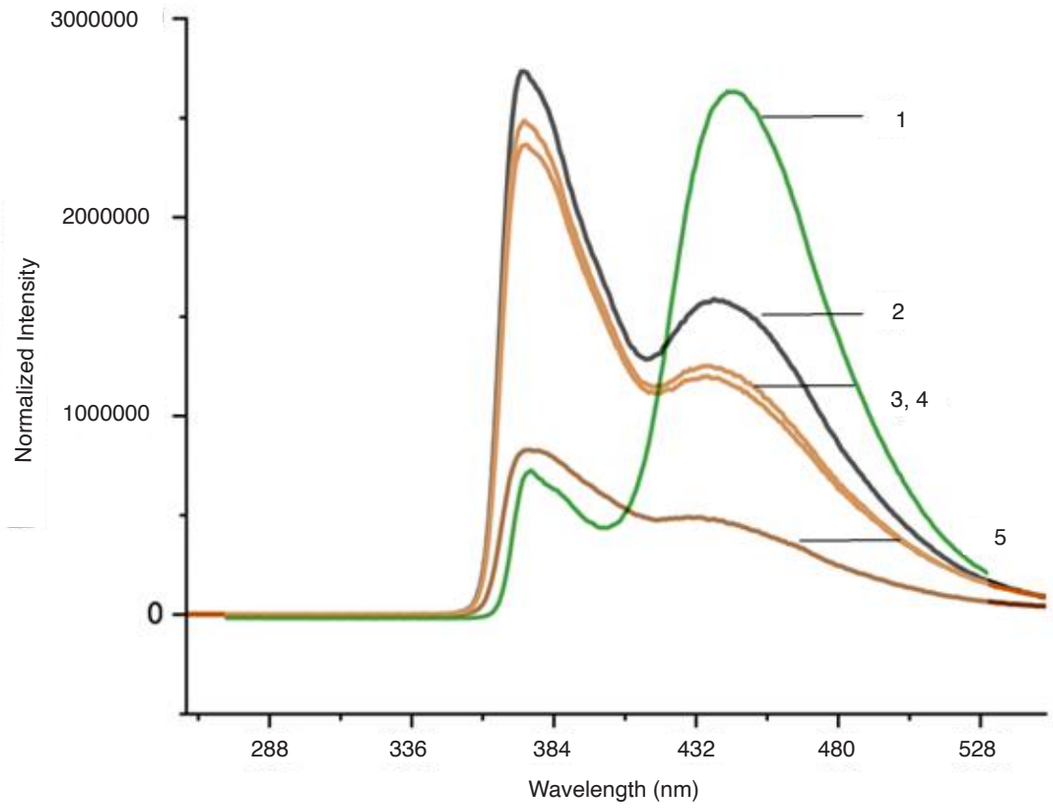

Figure 11- *Emission response on spiking alcoholic drinks with AMI (1) CRSA (2) Vodka (3) Whiskey (4) Rum (5) Wine*

olanzapine, clozapine. But with the addition of amisulpride, the fluorescence is quenched significantly. The quenching percentage of the various drugs compared to amisulpride is as shown in Figure 6. Prominent quenching is also visibly observed under the UV lamp as shown in Figure 7.

To understand the sensing properties more thoroughly the effect of concentration of the analyte on the fluorescence quenching of CRSA was studied (Figure 8). The fluorescence intensity steadily de-

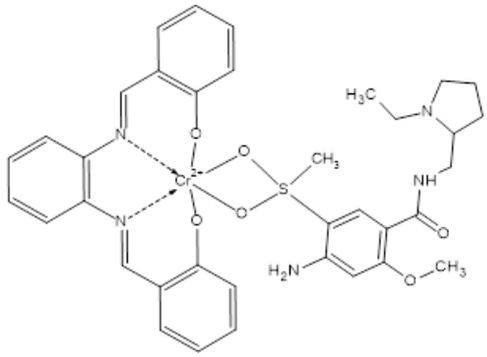

Figure 12- *CRSA- AMI complex after quenching*

creases with increasing concentration of amisulpride from 0 to 11.1 x 10-6 M. A good linear relationship is established as shown in Figure 9. In addition, the quenching efficiency is evaluated using the Stern-Volmer equation, F0/F=1+Ksv[M] where M is the concentration of amisulpride and Ksv is the Stern-Volmer quenching constant. The value of the Stern-Volmer constant is calculated to be 1.8 x 105 M-1 indicating a good quenching efficiency. The limit of detection for amisulpride is evaluated to be 1.6 x 10-6 M from the Stern-Volmer Plot.

3.3. Effect of pH on the sensing

To study the effectiveness of the developed method and its applicability to real situations the impact of pH on the sensing ability of CRSA was studied. As shown in the Figure 10, the quenching efficiency is affected in the alkaline medium (pH= 9.2, 9.9, and 10.6). Hence, we may conclude that for a forensic analysis, an alkaline matrix will have to be neutralized or acidified before using CRSA as to determine amisulpride.

3.4. Application in Real Samples

To study the applicability of the proposed method for the detection of AMI in toxicological and forensic samples; alcoholic drinks spiked with AMI were studied. The samples showed good quenching of fluorescence in the presence of CRSA without any sample preparation or isolation as shown in Figure 11.

3.5. Plausible mechanism

The SALP ligand does not show any fluorescence, probably due to flexible structure of the C=N which can undergo photo isomerization. But the complexation with Cr (III) causes rigidification of structure leading to suppression of movements, causing the molecule to have fluorescence properties. In the drugs that have been studied, AMI has a –SO2 group which is similar to a carboxylic moiety and it is known that Cr (III) has a strong affinity for carboxylic acid functional group [27]. Hence, the quenching proceeds via a static quenching mechanism forming a complex appear as Figure 12.

4. Conclusion

In the present work a fluorescent chemosensor is developed for the sensitive and selective detection of AMI. The method can be adapted for onsite detection since the quenching is easily visualized in a simple UV chamber without the use of any sophisticated instruments. The synthesis of the sensor is also simple and has a very low detection limit demonstrating its applicability in forensic toxicology cases. The on-site analysis is also advantageous as it is selective in the presence of other similar drugs and in presence of alcoholic beverages as well. The results do not vary with interference of drugs and beverages as well as change in pH.

Conflict of Interest

The authors declare that there is no conflict of interest.

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