DOI : [http://doi.org/10.22438/jeb/40/3\(SI\)/Sp-14](http://doi.org/10.22438/jeb/40/3(SI)/Sp-14)

Detection of ribavirin in environmental and biological samples via optical chemical sensor

Paper received: 17. 04. 2018

Revised received: 14. 11.2018

Accepted: 03.01. 2019

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Abstract

Aim: This study was conducted to determine the Ribavirin in Environmental and Biological Samples via Optical Chemical Sensor.

Methodology: In this paper, a new magnetic metal-organic framework $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ was synthesized with nano- Fe_3O_4 as metal center and a new Schiff base, which was synthesized from quinoline-2-formaldehyde and β -mercaptoethylamine, as ligand. This $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ was characterized by FITR, SEM and XRD. Its enzyme-like activity was characterized by the chromogenic substrate (H_2O_2 and TMB) and verified based on the Michaelis-Menten equation.

Results: The results showed that the synthesis of $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ was successful and it exhibited excellent enzyme-like activity. The $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ was successfully used for the detection of ribavirin in water samples as catalytic material. It showed good stability, accuracy and precision. This testing method features a graph, which plots concentration ($c_{\text{ribavirin}}$) on the x-axis and absorption A on the y-axis to create a linear equation. It shows a good linear relation ranging from $1 \times 10^{-4} \text{ mol l}^{-1}$ to $1 \times 10^{-6} \text{ mol l}^{-1}$, with a limit detection of $2 \times 10^{-7} \text{ mol l}^{-1}$. The mean recovery rates for spike recovery experiments varied from 92.0 % to 109 %.

Interpretation: A new Schiff base was synthesized from quinoline-2-formaldehyde and β -mercaptoethylamine. Then, a new magnetic metal-organic framework $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ was synthesized by it with nano- Fe_3O_4 as the metal center. The $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ was successfully used for the detection of ribavirin in water samples as catalytic material. It showed good stability, accuracy and precision.

Key words : Catalyst, Feed, Optical chemical sensor, Ribavirin

Citation: Wei, S. , L. Shao, S. Zhang, H. Cheng and W. Liang: Detection of ribavirin in environmental and biological samples via optical chemical sensor. *J. Environ. Biol.*, **40**, 497-508 (2019).

Introduction

Ribavirin, also known as virazole, RTCA and tribavirin, is a broad-spectrum antiviral drug (Wenying, 2010; Shaikh *et al.*, 2017). Researchers home and abroad have analyzed its risks and clinical data indicate that ribavirin has side effects (Hua *et al.*, 2006). Serious side effects include dissolution anemia, genotoxicity, reproductive toxicity, carcinogenicity and teratogenicity (Jingxian *et al.*, 2013; Cho, 2017; Ayaz *et al.*, 2018). As ribavirin is only used for human in clinic and lacks scientific basis and pharmacological research on animals. On March 22, 2006 the FDA published a final rule prohibiting the extra label use of adamantane and neuraminidase inhibitor classes of antiviral drugs in animals. As ribavirin is only approved for treating humans, it lacks scientific evidence and pharmacological studies on animals (Lei *et al.*, 2013; Wahab and Adzmi, 2017; Jamal *et al.*, 2018). There are few methods for the detection of ribavirin residues inside animals or in animal feeds, so it is necessary to find a rapid, simple and effective method for the detection of ribavirin.

At present, ribavirin is mainly detected by HPLC, UV spectrophotometry, capillary electrophoresis and HPLC-MS/MS (Xiaozhen, 2011; Wanzhen *et al.*, 2005; Fei and Li, 2011; Azizan *et al.*, 2017; Biswas, 2018). However, these methods require significant time for analysis, expensive instruments and skilled operators. Such high requirements hinder their further application.

Several reports are available on H_2O_2 as catalysis, both at home and abroad, including natural HRP, nano hydrogen peroxidase and metal-organic framework (Haijun *et al.*, 2007; Xiufang, 2014; Yang *et al.*, 2015; Qingyuan *et al.*, 2012; Dong *et al.*, 2015; Basheer *et al.*, 2017; Fahim and Sathi, 2018).

The metal-organic framework (MOF) are compounds consisting of metal ions or clusters coordinated to organic ligands to form multi-dimensional structures. They are a subclass of coordination polymers, with special feature that are often porous. MOF materials have many advantages, including porosity, large surface area and great catalysis (Kang, 2015; Ashik *et al.*, 2018). MOFs have drastically different catalytic activities depending on the framework present. The metal-organic framework centered with magnetic metal is easily formed through magnetic separation during catalytic separation; a prefabricated MOF with different new linkers may show different properties, such as hydrogen storage or selective catalysis (Fei, 2014; Yingya *et al.*, 2008; Ismail and Hanafiah, 2017).

Schiff base has drawn much attention since its creation due to its good performance as ligands to form coordination complexes with metal ions. It has been reported that Schiff base metal complexes can be used for the synthesis of enzyme inhibitors (Mingrong, 2013; Elmnifi *et al.*, 2018). Other researches

also published mercapto Schiff base and its transition metal complexes but reports on their enzyme-like activities are few (Shuai, 2013; Xu, 2018; Chidumayo, 2018).

In this paper, a new magnetic MOF $Fe_3O_4@Fe-ASB$ was synthesized with nano- Fe_3O_4 as its metal center and new Schiff base (synthesized from quinoline-2-formaldehyde and β -mercaptoethylamine) as ligand. This paper aims to develop a colorimetric sensor to measure the concentration of ribavirin in water and animal feeds, and is based on the oxidation of ribavirin with H_2O_2 , which leads to blue color of H_2O_2 +TMB system fading away. In this way, we would study the enzyme-like activity of newly synthesized MOF $Fe_3O_4@Fe-ASB$.

Materials and Methods

Instruments and reagents: UV-2600 Spectrophotometer (Shimadzu), D/MAX2200 X-ray Single Crystal Diffractometer (Rigaku), FTIR-8400 (Shimadzu), 1200 HPLC (Agilent Technologies), Leici PHS-3C pH Meter (Shanghai INESA Scientific Instrument Co., Ltd.) Ribavirin standard sample (Guangdong Zhaoqing Star Lake Biotechnology Co., Ltd.); iron (III) chloride hexahydrate, 3,3',5,5'-tetramethyl benzidine (TMB), thioglycolic acid (TGA), β -mercaptoethylamine (the above-mentioned reagents were analytical reagents, purchased from Shandong West Asia Chemical Industry Co., Ltd.), glacial acetic acid (HAc), sodium acetate trihydrate (NaAc), 25% ammonia water, 30% H_2O_2 (the above-mentioned reagents were all analytical reagents, purchased from Guangzhou Chemical Reagent Factory), ethylene glycol AR (EG) (from Tianjin Guangcheng Chemical Reagent Co., Ltd.), acetonitrile AR (Xilong Chemical Co., Ltd.), n-hexane AR (Tianjin Kermel Chemical Reagent Co., Ltd.), quinoline-2-formaldehyde AR (Shanghai Jingchun Biochemical Technology Co., Ltd.), trichloroacetic acid AR (Shanghai Lingfeng Chemical Reagent Co., Ltd.). The water used in the experiment was purified water; the feeds were pig feeds from Zhaoqing city (No. 951, 923, 902 and 983). The Water Sample No. 1 was collected from the Xijiang River.

Synthesis of magnetic metal-organic framework: Synthesis of Schiff base from quinoline-2-formaldehyde and β -mercaptoethylamine (ASB): A 0.3930 g of quinoline-2-formaldehyde was weighed and added to a 5 ml beaker, which contained absolute ethyl alcohol, after its full dissolution, the solution was transferred into a 50 ml round-bottom flask. Then 0.1938 g β -mercaptoethylamine was weighed and dissolved it in 5 ml absolute ethyl alcohol. The solution was added dropwise into a flask containing quinoline-2-formaldehyde. After injection, 1 ml glacial acetic acid was added. The reaction took 4hr under magnetic stirring. The temperature was maintained at 60 °C. After the reaction, the product was cooled to room temperature. Then it was dried in vacuum oven at 50 °C. After the solvent evaporated completely, the mixture was washed several times with absolute

ethanol, and dried at 50 °C vacuum drying oven. The product was a blood red viscous solid.

Synthesis of nano-Fe₃O₄: A 3.46 g FeCl₃ was dissolved in 70 ml ethylene glycol solution under continuous stirring. After its complete dissolution, 7.66 g NaAc was added and dissolved under stirring for another 30 min. After its complete dissolution, the reaction liquid was divided evenly into two parts, and moved into a 50 ml stainless steel autoclave. The reaction lasted for 8 hr at 200 °C in oven. After the reaction, the product was cooled to room temperature. Then it was taken out of the oven and washed alternately with absolute ethanol and distilled water 5 times. Then, the product was dried at 50 °C and placed in sample tubes for further use.

Synthesis of Fe₃O₄@Fe-ASB: A 1.00 g of nano-Fe₃O₄ was prepared and placed in a beaker, and then 50 ml of absolute ethanol was added for ultrasound aid dispersion, which lasted 20 min. After dispersion and magnetic separation, 20 ml of 2.9 m mol l⁻¹ TGA was added for ultrasound treatment for 20 min. After completion of ultrasound dispersion, the product was put in a shaker for 1 hr. After that, the apparatus was left overnight. The product was washed with absolute ethanol and distilled water respectively, and then 40 ml of 10 mmol l⁻¹ FeCl₃ solution was added. The reaction lasted for 1 hr in water bath at 70 °C. After reaction, the product was washed twice with absolute ethanol and distilled water, and then 40 ml of 10 mmol l⁻¹ ASB solution was added in the product. The reaction lasted for 1 hr at 70 °C and its product was washed three times with absolute ethanol and distilled water, respectively, and dried in a vacuum drying oven for 24 hr at 50 °C. The final product was a bulky black solid, known as magnetic MOF catalyst.

Study of enzyme-like activity of Fe₃O₄@Fe-ASB and analysis of catalytic kinetic: 10 mg of Fe₃O₄@Fe-ASB and 2 ml of 0.1 mol l⁻¹ HAC-NaAc was taken in two test tubes (pH 4). After vortexing for 10 sec, 100 µl of 8 mmol l⁻¹ TMB solution; and in 2nd test tube 100 µl of H₂O₂ and 100 µl of 8 mmol l⁻¹ TMB solution was added; then we take another test tube and added 100 µl of H₂O₂ solution and 100 µl of 8 mmol l⁻¹ TMB solution. After 10 min, absorbance was read at 652 nm.

Detection of ribavirin: A 10 mg Fe₃O₄@Fe-ASB was added in a test tube first, and then 2 ml of 0.1 mol l⁻¹ HAC-NaAc (pH=4) was added. After vortexing for 10 sec, 100 µl of H₂O₂ and 100 µl of ribavirin solution was added. The solution was incubated for 10 min. 100 µl of 8 mmol l⁻¹ TMB solution was added to terminate the color for 10 min, the absorption of each was measured at 652 nm with UV detection. Both incubation and reaction took place at 35 °C.

Pretreatment of Water sample: The water samples were collected from Zhaoqing part of Xijiang River and boiled for 1 min and filtered for detection.

Feed sample: Before treatment, the feed sample was dried in 60 °C vacuum oven for 4 hr. Then, 2 g of feed sample was taken a centrifuge tube and 10 ml of acetonitrile-1 % trichloroacetic acid (70:30) was added. After vortexing for 1 min, the solution was centrifuge for 5 min at 10000 r min⁻¹. Then clear supernatant was transferred into another 50 ml centrifuge tube and 10 ml of n-hexane was added. After vortexing for 30 sec, the solution was centrifuged for 5 min at 10000 r min⁻¹. The upper solution was discarded, and the lower solution was adjusted to pH 8.5±0.1 with ammonia water. After centrifuge for 5 min, the eluent was filtered for further detection (Kai *et al.*, 2014). HPLC detection conditions were as follows: the column was ODS-BP column (4.6 × 250 mm, 5 µm), mobile phase was a solution of methanol: water (adjusted to pH=4 with sulfuric acid) = 12:88(V/V), the flow rate was 0.8 ml·min⁻¹. Three types of wavelengths were adopted, 207 nm for ribavirin, 254 nm for carnine, 260 nm for uridine and 20 µl for the sample.

Results and Discussion

Reaction mechanism: Fig. 1, 2, 3 shows the reaction mechanism. The ribavirin adduct LC-MS and ribavirin LC-MS are shown in Fig. 5 and Fig. 4. The molecular ion peak was at 245 m z⁻¹. The peak of ribavirin sodium salt dihydrate was the number remained same after binding, indicating that the adduct reaction was incomplete. Before binding, the peak at 268 m z⁻¹ was high, while peak at 303 m z⁻¹ was low. In contrast, after binding, the peak at 268 m z⁻¹ fell, while the peak at 303 m z⁻¹ rose. The peak at 268 m z⁻¹ was the molecular ion peak of ribavirin sodium salt, while peak at 303 m z⁻¹ was the molecular ion peak of adduct formed from ribavirin and H₂O₂ with an increase of molecular weight equaling 34.

Characterization of Fe₃O₄@Fe-ASB: Fig. 6 shows the characterization of ASB. The peak at 301 nm of UV spectrum of quinoline-2-formaldehyde was the absorption peak of C=O bond. In the reaction, the C=O bond reacted with NH₂ and formed a C=N double bond, so there no peak at 301 nm. A new peak appeared, compared with the UV spectrum of quinoline-2-formaldehyde. It was the characteristic absorption peak of C=N double bond. Due to formation of ASB from quinoline-2-formaldehyde and β-mercaptoethylamine, two peaks at 203 nm and 231 nm were red-shifted.

The above graph was infrared band of ASB (A). The peak at 3400.98 cm⁻¹ was sharp, while the characteristic absorption peaks at 2300.58 cm⁻¹ and 2062.95 cm⁻¹ were weak. They were the characteristic absorption peaks of S-H bond. The peaks at 2937.69 cm⁻¹ and 1380.09 cm⁻¹ were due to stretching vibrations of C-H bond in methylene; the peak at 1659.16 cm⁻¹ was the characteristic absorption peak of C=C-C=C bond and C=N bond; the peak at 916.00 cm⁻¹ was due to stretching vibrations of C-N bond, whereas peak at 620.59 cm⁻¹ resulted from out of plane bending of unsaturated olefin C-H.

Fig. 1 : Ribavirin adduct reaction Ribavirin 268 m/z Ribavirin adduct 303 m z⁻¹.

Fig. 2 : Synthesis route of catalyst Nano-Fe₃O₄ TGA modified nano-Fe₃O₄.

Fig. 3 : Principle of ribavirin detection with colorimetric method Nano-Fe₃O₄.

As shown in the infrared spectrum of Fe₃O₄@Fe-ASB and Fe₃O₄, an obvious absorption peak appeared at 591.5 cm⁻¹ of Fe₃O₄ spectrum. It was the characteristic absorption peak of Fe₃O₄. The characteristic absorption peak of Fe₃O₄ lies in 474.10 cm⁻¹ of spectrum of Fe₃O₄@Fe-ASB. After the magnetic MOF wrapped the Fe₃O₄, the characteristic absorption peak of Fe₃O₄ blue-shifted. The absorption peak at 1084.01 cm⁻¹ of Fe₃O₄@Fe-ASB spectrum was the ligand peak of Fe-ASB.

Fig. 8 shows the SEM images of Fe₃O₄@Fe-ASB. The Fe₃O₄@Fe-ASB had high dispersion and its particles were uniform in size and round in shape. The diameter of Fe₃O₄@Fe-ASB was about 280 nm, as shown in Fig. 8(B).

Fig. 9 shows the XRD diagram of Fe₃O₄@Fe-ASB. The diffraction peaks of self-made XRD diagrams of Fe₃O₄ (a) and Fe₃O₄@Fe-ASB (b) were consistent. So, did the diffraction peaks of self-made Fe₃O₄ (a) and JCPDS 65-3107, corresponding to (220), (311), (400), (422), (511), and (440) crystal planes of Fe₃O₄, respectively. Although the diffraction peaks of Fe₃O₄@Fe-ASB (b) and Fe₃O₄ (a) were consistent, their intensity and width were not same due to the magnetic MOF that wrapped the Fe₃O₄@Fe-ASB. However, such magnetic MOF did not affect the original crystal structure of Fe₃O₄ (Donglan, 2013).

Study of enzyme-like activity of Fe₃O₄@Fe-ASB: Fe₃O₄@Fe-ASB has enzyme-like activity, and acts as a catalyst for H₂O₂, which oxidizes its substrate to develop color. In the absence of catalyst, such process cannot be realized. Better enzyme-like activity of the catalyst will result in faster catalytic reaction and more obvious rendering of color. To study the enzyme-like activity of Fe₃O₄@Fe-ASB, a comparative experiment was conducted. Fig. 10 shows the presence of only H₂O₂ and TMB substrate, the

Fig. 4 : Ribavirin LC-MS.

solution was colorless and transparent, indicating that the H_2O_2 in the system did not oxidize the substrate TMB and develop color. In the presence of catalyst and TMB substrate, the solution was light blue because a trace amount of oxygen had dissolved in the solution. So in the presence of catalyst, oxygen reacted with H^+ ions and produced HO_2^- , which then oxidized the TMB substrate and rendered color. In the presence of catalyst, H_2O_2 and substrate TMB, the solution showed transparent blue because a large amount of H_2O_2 had oxidized TMB colour substrate and developed the color (Yang *et al.*, 2015; Nawaz *et al.*, 2018). Coloration was obvious, which proved that $\text{Fe}_3\text{O}_4@\text{Fe-AS}$ has a good enzyme-like activity.

Many researches have reported (GAO Q. *et al.*, 2011) the

catalytic activity of nano- Fe_3O_4 . Some researchers demonstrated that certain metal-organic frameworks had catalytic activity. To understand whether the combination of both nano- Fe_3O_4 and magnetic metal-organic frameworks can increase catalytic effects, a comparative experiment was conducted. The result is shown in Fig. 11.

The absorbance of both MOF (a) and Fe_3O_4 (b) was lower than that of $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ (c), which approved the synergy of Fe_3O_4 and MOF. Therefore, $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ showed better enzyme-like activity than the other two. Fig. 12 showed that the curve of absorbance varied with the amount of catalyst. Fig. 12 shows that the absorbance increased as the amount of catalyst was increased, but with decreased effect, indicating that the

Fig. 5 : Ribavirin adduct LC-MS.

maximum reaction rate would reach a balance as the amount of catalyst was increased. After taking all factors into consideration, the experiment used 10 mg catalyst as the optimal catalyst amount.

Analysis of catalytic kinetic of $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$: To further understand the enzymatic activity of $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ in simulating enzyme, the K_m of H_2O_2 and TMB substrate in $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ was measured, using same data of self-made Fe_3O_4 as a comparing group. Michaelis constant (K_m) is a enzymes constant affected by reaction conditions, properties of substrates

and to enzymes. Smaller the K_m , greater is the affinity of enzymes to substrates, and to reach the maximum reaction rate becomes quicker and easier. On the contrary, greater the K_m , value smaller is the affinity of enzymes to substrates, and to reach the maximum reaction rate becomes slower and more difficult. The Michaelis constant is usually calculated with double-reciprocal plot of the Michaelis-Menten equation.

$$v = \frac{\Delta c}{\Delta t} = \frac{\frac{\Delta A}{\epsilon b}}{\Delta t} \quad (1)$$

Fig. 6 : UV spectra of quinoline-2-formaldehyde (a) and ASB (b)

Fig. 7 : Infrared spectra of ASB (A), Fe_3O_4 @Fe-ASB (a) and Fe_3O_4 (b).

Fig. 8 : SEM images of Fe_3O_4 @Fe-ASB

Fig. 9 : XRD diagrams of Fe_3O_4 (a) and Fe_3O_4 @Fe-ASB (b).

Fig. 10 : Study of the enzyme-like activity of $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ H_2O_2 +TMB (a); $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ +TMB (b); $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ + H_2O_2 +TMB (c).

Fig. 11 : Comparison of enzyme-like activity of different catalysts Metal-organic framework (a); Fe_3O_4 (b); $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ (c).

Fig. 12 : Curve of absorbance varying with the amount of catalyst 2 mg (a); 4 mg (b); 6 mg (c); 8 mg (d); 10 mg (e).

In Equation (1), ΔA is the variation of absorbance in Δt , the molar absorptivity of the product from oxidation of TMB substrate, known as ϵ , at 652 nm: $\epsilon_{652} = 39000 \text{ M}^{-1}\text{cm}^{-1}$, the thickness of colorimetric pond, known as b , was 1 cm.

After the initial reaction rate was obtained, according to the double-reciprocal plot of Michaelis-Menten equation:

$$\frac{1}{v} = \frac{K_m}{V_m} \cdot \frac{1}{[S]} + \frac{1}{V_m} \quad (2)$$

In Equation (2), v is the initial reaction rate; V_m is the maximum reaction rate; S is the substrate concentration; and K_m is the Michaelis constant.

Fig. 13: Michaelis-Menten equations for $\text{Fe}_3\text{O}_4\text{@Fe-ASB}$ catalyzing H_2O_2 (left) and substrate TMB (right).

Table 1 : K_m and V_m for different catalysts and substrates

Catalyst	Substrate	K_m (mM)	V_m ($M \cdot s^{-1}$)
Fe_3O_4	H_2O_2	0.324	3.921×10^{-8}
Fe_3O_4	TMB	0.038	7.750×10^{-8}
$Fe_3O_4@Fe-ASB$	H_2O_2	0.084	4.078×10^{-8}
$Fe_3O_4@Fe-ASB$	TMB	0.020	5.780×10^{-8}
HRP[20]	H_2O_2	3.70	8.710×10^{-8}
HRP[20]	TMB	0.434	1.000×10^{-7}

Table 2: Recovery rate and standard error of water samples (n=3)

Sample	Measured value (mM)	Addition amount (μM)	Average recovery amount (μM)	Recovery rate (%)	Standard error (%)
Water sample	ND	5.00	4.60	92.0	1.1
		10.0	10.9	109	2.7
		50.0	5.23	105	3.1
Feed sample	ND	5.00	5.20	104	1.1
		10.0	9.80	98.0	1.8
		50.0	49.3	98.6	1.6

ND: not detected

Catalyst was weighed in its precise amount, and changed substrate concentration, with other conditions remained the same, to draw a graph of absorbance vs. time at 652 nm. The time for catalytic reaction of substrate was 120 sec and 240 sec. The concentration of products at different time was calculated based on the Lambert's Law. According to double-reciprocal plot of Michaelis-Menten equation, two linear equations were made with $1/c(H_2O_2)/mM^{-1}$ as X-axis and $1/v/10^{-8}s \cdot M^{-1}$ as Y-axis. Then Michaelis constant of K_m was calculated and the maximum reaction rate of V_m based on the regression equation. The intercept on X-axis was the absolute value of reciprocal of K_m , and intercept on Y-axis was V_m . Fig. 13 shows that Michaelis-Menten equation for $Fe_3O_4@Fe-ASB$ catalyzing H_2O_2 is as follows: $y=0.0205x+2452$, in which $K_m=0.0836$ mM and $V_m=4.078 \times 10^{-8} M \cdot s^{-1}$. Fig. 13 also shows the Michaelis-Menten equation for $Fe_3O_4@Fe-ASB$ catalyzing TMB: $y=0.0035x+0.173$, in which $K_m=0.0202$ mM and $V_m=5.780 \times 10^{-8} M \cdot s^{-1}$ (Table 1).

Table shows that whether the substrate was H_2O_2 or TMB, the K_m of $Fe_3O_4@Fe-ASB$ was lower than that of Fe_3O_4 and HRP. The affinity of $Fe_3O_4@Fe-ASB$ to substrate was greater than that of Fe_3O_4 and HRP. With equal substrate concentration, $Fe_3O_4@Fe-ASB$ reached the maximum reaction faster than Fe_3O_4 and HRP. Therefore, $Fe_3O_4@Fe-ASB$ showed better enzymatic activity.

Optimization of detection conditions: Optimization of pH:

Fig. 14 shows that the color of buffer solution was most intense at pH 3. As pH increased, the intensity of color decreased. The absorbance curve leveled out slightly at pH 4 and then fell again. At pH 5, the color slowly disappeared because under neutral or

alkaline conditions H_2O_2 exhibits reducing property instead of oxidizing property, even catalyst are not able to oxidize the substrate TMB and develop color. Fig. 14 shows that pH 3 the most favorable condition to develop color. However, at pH 3, Fe^{3+} may dissolve into the solution from $Fe_3O_4@Fe-ASB$, and there is a high concentration of H^+ , resulting in the interference, which leads to unstable coloration of solution. Given that, the experiment was carried out with 0.1 mol l^{-1} HAC-NaAc buffer solution at pH 4.

Optimization of incubation time: Fig. 15 shows the incubation time, i.e., the reaction time of H_2O_2 and ribavirin. Higher H_2O_2 was consumed, less obvious the color was for the system. Besides, the more complete a reaction, the higher accuracy it achieves. The complete reaction of ribavirin and H_2O_2 has been found to occur at 10min. Due to time constraints and the fact that H_2O_2 may decompose under long exposure, we select the 10min incubation time for the experiment.

Optimization of incubation temperature: Curve A shows the effect of temperature on the absorption of ribavirin. From Fig. 16 we can see that higher incubation temperature leads to lower absorbance. There were two possible reasons: First, the reaction between ribavirin and H_2O_2 tended to complete as incubation temperature increased; Second, H_2O_2 decomposed as incubation temperature increased. Curve B shows the effect of incubation temperature on absorbance of H_2O_2 oxidation substrate. From Curve B we can see that the coloration of H_2O_2 was most visible at 35 °C. There were two possible explanations: either at 35 °C the H_2O_2 oxidation substrate tended to complete, resulting in most obvious coloration, or at 35 °C the reaction between H_2O_2 and ribavirin was incomplete, and the remaining H_2O_2 oxidized more

Fig. 14 : Effect of buffer solution pH on the system.

Fig. 15 : Effect of incubation time on the absorbance.

Fig. 16 : Optimization of incubation temperature (A) and H_2O_2 coloration (B).

substrate and developed color. Based on observation from Curve A and Curve B, the difference reached maximum at 35°C, indicating that the reaction of ribavirin and H_2O_2 was most complete and decomposition of H_2O_2 was not significant. Therefore, the incubation temperature of 35 °C was selected for incubation and coloration processes.

Detection of ribavirin: Linear equation and detection limit: A straight-line equation was drawn for standard concentration of ribavirin solution in water samples (X-axis) and absorbance (Y-axis), which showed a good linearity in between $1 \times 10^{-4} \text{ mol l}^{-1}$ ~ $1 \times 10^{-6} \text{ mol l}^{-1}$. The linear equation was $y = -1160.3x + 0.1517$, and the detection limit was $2 \times 10^{-7} \text{ mol l}^{-1}$.

The standard ribavirin solution of $2 \mu\text{mol l}^{-1}$, $5 \mu\text{mol l}^{-1}$, $8 \mu\text{mol l}^{-1}$, $20 \mu\text{mol l}^{-1}$ and $80 \mu\text{mol l}^{-1}$ were prepared respectively, with blank feed samples obtained after treatment. In the

experiment, 10 mg of $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ and $5 \times 10^{-4} \text{ mol l}^{-1} \text{H}_2\text{O}_2$ were used. A standard curve was drawn for standard ribavirin solution concentration in blank feed samples (X-axis) and absorbance (Y-axis), which showed a good linearity between $1 \times 10^{-4} \text{ mol l}^{-1}$ ~ $1 \times 10^{-6} \text{ mol l}^{-1}$. The linear equation was $y = -4283.9x + 0.3659$, and the detection limit was $2 \times 10^{-7} \text{ mol l}^{-1}$.

Precision and accuracy: The water and feed samples in the experiment were determined after simple pretreatment. Three ribavirin solutions with different concentration were used to conduct an additional experiment, which was repeated for three times to obtain the recovery rate and standard error (Gao *et al.*, 2007; Tunggolou and Payus, 2017). This is to evaluate the accuracy and precision of the method. In the experiment, the blank treatment solution was used as matrix to eliminate interference. The results are shown in Table 2. The recovery rate was about 92.0 %~109 % with standard error at 1.1 %~3.1 %,

Fig. 17: Standard curve for determination of ribavirin in water samples $2 \mu\text{mol l}^{-1}$ (S1); $5 \mu\text{mol l}^{-1}$ (S2); $8 \mu\text{mol l}^{-1}$ (S3); $20 \mu\text{mol l}^{-1}$ (S4); $100 \mu\text{mol l}^{-1}$ (S5).

showing that the method had high accuracy and precision (Table 2).

Based on UV spectrophotometry, a method for indirect measure of ribavirin in water samples via H_2O_2 oxidation of substrate was designed. It was successfully used for the determination of ribavirin in feed samples and showed the character of stability, accuracy and precision. In this paper, a new Schiff base that can strongly coordinate with metals was synthesized. It was used as ligand to form a new magnetic metal-organic framework $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$, together with nano- Fe_3O_4 as metal center. The $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ has good affinity to substrates and shows catalytic property. Due to its magnetic core structure, it shows good, stability and strong catalytic activity in the catalytic process. In future, it can be produced in large scale and applied to a wide range of analytical samples. The pretreatment method used in the experiment is simple, non-toxic, and can be carried out at room temperature without the need for large-scale instruments, indicating the broader potential of applications for the method in monitoring, quality control and sample analysis.

In this paper, a new Schiff base that can strongly coordinate with metals was synthesized. It was used as the ligand to form a new magnetic metal-organic framework $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$, together with nano- Fe_3O_4 , as the metal center. The $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ has good affinity to substrates and shows catalytic property. The $\text{Fe}_3\text{O}_4@\text{Fe-ASB}$ was successfully used for the detection of ribavirin in water samples as catalytic material. It showed good stability, accuracy and precision.

Acknowledgments

This work is financially supported by the fund of Guangxi Key Laboratory of Green Processing of Sugar Resources (GXTZY201701), the fund of Key Laboratory for Processing of

Fig. 18: Standard curve for detection of ribavirin in feed samples $2 \mu\text{mol l}^{-1}$ (S1); $5 \mu\text{mol l}^{-1}$ (S2); $8 \mu\text{mol l}^{-1}$ (S3); $20 \mu\text{mol l}^{-1}$ (S4); $80 \mu\text{mol l}^{-1}$ (S5).

Sugar Resources of Guangxi Higher Education Institutes (2016TZYKF01) and high levels of innovation team and excellence Scholars Program in colleges of Guangxi.

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