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Yue Luo

Guangxi Normal University

Dan Li

Guangxi Normal University

Chongning Li

Guangxi Normal University

Guiqing Wen

Guangxi Normal University

Aihui Liang

ahliang2008@163.com

Guangxi Normal University

Zhiliang Jiang

Guangxi Normal University

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A selective resonance Rayleigh scattering method for trace vitamin B2 based on molecularly imprinted nanosilver probe

Yue Luo^{1,2}, Dan Li^{1,2}, Chongning Li^{1,2*}, Guiqing Wen^{1,2}, Aihui Liang^{1,2*}, Zhiliang Jiang^{1,2}

¹ Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education, Guilin 541004, China;

² Guangxi Key Laboratory of Environmental Pollution Control Theory and Technology, Guilin 541004, China.

Corresponding author E-mail address: ahliang2008@163.com.

Abstract: Using nano-Fe₃O₄ as the matrix, vitamin B2 (VB₂) as the template molecule, and AgNO₃ as the precursor for the molecularly imprinted (MI) layer, a new nanosilver probe (Fe₃O₄@AgNP) for detecting VB₂ was prepared by the photochemical procedure. The probe was characterized by molecular spectroscopy, electron microscopy, energy dispersive spectroscopy and magnetic analysis. It exhibits a strong surface plasmon resonance Rayleigh scattering (RRS) peak at 380 nm. When VB₂ binds to the Fe₃O₄@AgNP probe, a strong RRS-energy transfer (RRS-ET) effect was observed, resulting to a decrease in RRS signal. Thus, a new, simple, selective and sensitive MI-AgNP RRS method was established, with a linear range of 5.0–120 nmol/L and a detection limit of 1.7 nmol/L VB₂. This method has been used to determine the VB₂ content in milk samples, with a relative standard deviation (RSD) of 0.23–1.8 % and a recovery of 96.34–107.85 %.

Keywords: vitamin B2; magnetic Fe₃O₄; surface molecularly imprinted silver nanoprobe; resonance Rayleigh scattering.

1. Introduction

Molecularly imprinted polymers (MIP) are three-dimensional network structures obtained through a polymerisation reaction in which functional monomers and cross-linking agents are thoroughly mixed with template molecules in a suitable solvent under the action of an initiator [1]. After washing away the template molecules, ‘imprint’ sites with specific structures and functions remain, which can specifically bind to target molecules to achieve selective recognition of molecules [2]. The preparation method of surface molecularly imprinted polymers originates from traditional molecular imprinting technology. Compared with molecularly imprinted polymers, surface molecularly imprinted polymers (SMIP) mainly form imprinting sites on the surface layer of the material, resulting in a high density of imprinting sites on the surface layer, thereby improving the binding capacity and separation efficiency of target molecules [3,4]. It was used in biochemical sensors, drug delivery systems, environmental monitoring, separation and purification, due to their excellent selectivity and stability. For example, Liu et al prepared magnetic MIP ($\text{Fe}_3\text{O}_4@\text{Ag}@\text{MIP}$) probes with surface-enhanced Raman scattering (SERS) activity by combining MIPs with magnetic nanoparticles for the ultra-sensitive, label-free detection of the illegal additive sibutramine, with a detection limit of 1.0×10^{-9} mol/L [5]. Using nano SiO_2 as molecularly imprinting layer, a SERS quantitative analysis method was reported for detection of trace malathion [6]. Ren prepared an $\text{Ag}@\text{MIP}$ SERS probe for the target molecule, and its Raman signal intensity is significantly higher than that of bare silver microspheres, with a detection limit as low as 10^{-9} mol/L [7]. However, SMIP requires multiple reagents such as monomers, crosslinking agents, initiators, and pore-forming agents, making the preparation process relatively complex. Therefore, exploring new, simple, and rapid molecular imprinting technologies, such as simple, stable and biocompatible precious metal imprinted layer, is of great significance. To our knowledge, there have been no reports on the construction of surface molecularly imprinted nanosilver probes for the detection of vitamin B2 (VB_2) using resonance Rayleigh scattering (RRS) technique.

RRS is a spectral analysis technique based on elastic Rayleigh scattering [8]. When the frequency of the excitation light is close to the resonance frequency of the nanoparticle, the intensity of the scattered light increases significantly, enabling RRS to detect trace substances [9-12]. RRS was combined with gold, silver, and other nanoprobe to enhance detection sensitivity [13]. Wen et al

proposed a self-catalytic oxidation mechanism, namely a novel nanocatalytic reaction involving AgNP-H₂O₂, using AgNP as a nanoprobe to develop a simple, sensitive, and selective RRS method for determining H₂O₂ in the range of 2.0×10^{-8} to 8.0×10^{-5} mol/L [14]. Shu et al utilized liquid crystal loaded Ag nanoparticles (LC@AgNPs) as signal amplification catalysts and combined them with specific aptamers as recognition elements to construct a highly sensitive and selective three-mode molecular spectral analysis platform for the efficient detection of inorganic pollutants such as Pb²⁺, Cd²⁺, Hg²⁺, and As³⁺ [15]. Li et al prepared ferrocene-doped polystyrene nanospheres sol-gel and constructed a SERS/RRS/absorption (Abs) trimode detection platform for Pb²⁺ [16]. To expand the range of detection substances, a RRS-energy transfer (RRS-ET) method was proposed [17-21]. It refers to the process of transferring energy from a nanoparticle donor to another molecule acceptor with absorption. When the RRS spectrum of the donor overlaps with the absorption spectrum of the acceptor molecule, the donor may transfer its RRS energy to the acceptor molecule. Liao et al reported a RRS-ET method for glyphosate using gold nanosurface molecularly imprinted polyacrylamide probe as donor [17]. Qin et al prepared doped-B carbon dots as donor probe and applied to determine trace phenol by RRS-ET [18].

VB₂ acts as cofactors, participating in energy production, red blood cell formation, and various enzyme-catalyzed reactions. It also aids in the metabolism of other vitamins and maintains the health of mucous membranes, skin, eyes, and the nervous system [22]. In order to prevent and treat VB₂ deficiency, the human body needs to consume a certain amount of VB₂. Currently, the analytical methods that have been proposed include fluorescence [23], microbiological [24], spectrophotometric [25] and amperometric [26] methods. For example, Xie et al developed a quinoline-pyridine combination chemical sensor modified with tetraphenylethylene. After chelating Cd²⁺, the chemical sensor exhibited high selectivity and sensitivity to VB₂ and folic acid [27]. Bindu et al synthesized titanium dioxide nanoparticles, and conducted electrochemical analysis of VB₂ using differential pulse voltammetry. The study investigated the effects of parameters such as pH and scan rate on detection performance, with VB₂ concentrations ranging from 0.2 μM to 3.4 μmol/L. The limit of detection (LOD) was 0.28 μmol/L, and the limit of quantification was 0.45 μmol/L [28]. However, some methods are costly, involve complex sample preparation, and are time-consuming. These methods cannot meet the requirements of analysis. This has prompted researchers to explore and

develop simple, sensitive, selective and practical methods. This paper investigates a facile Fe_3O_4
 $_{50\text{nm}}@\text{AgNP}$ spectral probe with specific recognition capabilities and, based on the RRS-ET effect,
develops a new RRS analytical method for the rapid, simple, sensitive, and selective detection of
 VB_2 .

2. Experimental

2.1 Instruments

Hitachi F-7000 Fluorescence Spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan); Microwave oven (Hefei Rongshida Small Appliances Co., Ltd., Hefei, China); TU-1901 Dual-Beam UV-Vis Spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China); N15UV Ultra-Pure Water Machine (Shanghai Yidian Scientific Instrument Co., Ltd., Shanghai, China); KQ3200DD CNC Ultrasonic Cleaning Machine (Kunshan Ultrasonic Instrument Co., Ltd., Nanjing, China); FB224 Automatic Internal Calibration Electronic Analytical Balance (Shanghai Yangguang Hengping Scientific Instrument Co., Ltd., Shanghai, China); RS-GB13A light wave furnace (Hefei Hefei Rongshida Small Appliances Co., Ltd., Hefei, China); DHG-9023A Electric Heating Constant Temperature Forced Air Drying Oven (Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China) were used.

2.2 Reagents

Fe_3O_4 powder(50 nm, 100 nm, 200 nm, North China Science and Technology Metal Materials Co., Ltd., Tianjing, China); Silver nitrate (AgNO_3 , Xilong Science Co., Ltd., Guangzhou, China); VB_2 ($\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_6$, Shanghai Maclin Biochemical Technology Co., Ltd.); sodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, Xilong Science Co., Ltd., Guangzhou, China); 30% hydrogen peroxide (H_2O_2 , Xilong Science Co., Ltd., Guangzhou, China); sodium borohydride (NaBH_4 , Xilong Science Co., Ltd., Guangzhou, China); methanol (CH_3OH , Guangdong Guanghua Technology Co., Ltd., Guangzhou, China); glacial acetic acid (CH_3COOH , 99.5%, Sichuan Xilong Science Co., Ltd., Guangzhou, China) were used. All reagents were of analytical grade, and the experimental water was pure water.

2.3 Preparation of Fe_3O_4 $_{50\text{nm}}@\text{AgNP}$

In an Erlenmeyer flask, 44 mL of pure water was added along with 35 mg of 50 nm Fe_3O_4 .

Under stirring conditions, 2.0 mL of 0.01 mol/L AgNO₃ was added sequentially, followed by 15.1 mg of VB₂, 3.0 mL of 0.1 mol/L citrate, 0.6 mL of 10 mol/L H₂O₂, and 0.60 mL of 100 mmol/L NaBH₄. The mixture was stirred for 5 min, heated in a light wave furnace for 10 min that set the temperature to 250°C, and then separated. An orange-red solution was obtained, and a magnet was used to adsorb and collect the solid powder. The solid powder was washed with 10 mL of methanol-acetic acid solution (methanol:acetic acid 9:1 v/v) in an ultrasonicator for 10 min to elute the template molecules until no UV-visible absorption peaks were detected in the eluent. Finally, the obtained solid powder was dried in a 60°C oven for 5 h to obtain Fe₃O₄ 50nm@AgNP. It was prepared a 0.10 g/L Fe₃O₄ 50nm@AgNP nanosol with water by ultrasonic 15 min. The non-molecularly imprinted polymer (Fe₃O₄ 50nm@AgNP_N) was prepared without VB₂, according to above operations.

2.4 Procedure

Different volumes of 5.0 µmol/L VB₂ were added to 5.0 mL test tubes. Then, 750 µL of 0.10 g/L Fe₃O₄ 50nm@AgNP and 100 µL of pH acetate buffer solution were added to each test tube, and the mixtures were mixed thoroughly. The solutions were diluted with pure water to 2.0 mL and allowed to sit for 15 min. A fluorescence spectrophotometer was used to perform a simultaneous scan of the system to obtain the RRS spectrum. The RRS intensity of the solution was measured at 380 nm, with a solution containing no VB₂ used as the blank (I₀). ΔI was calculated as I₀ – I.

3. Results and discussions

3.1 Analysis principle

Using Fe₃O₄ as the core, VB₂ as the template molecule, and AgNO₃ as the imprinting layer precursor, the mixture is stirred to ensure thorough mixing. A reducing agents including NaBH₄ and H₂O₂ is then added to reduce the Ag⁺ to form Ag nanolayer (AgNL), and AgNL-VB₂ composite by means of Ag-N and Ag-O coordination and intermolecular forces including hydrophobic interaction. After elution, a Fe₃O₄ 50nm@AgNP probe with VB₂ imprinted sites was obtained. The relative saturation magnetization of Fe₃O₄@AgNP is lower than the Fe₃O₄. It indicates that the AgNL cover the surface of Fe₃O₄. In the RRS-energy transfer (RRS-ET) system, it refers to the process of transferring energy from one molecule or nanoparticle (donor) to another molecule or particle (acceptor). When the RRS spectrum of the donor particle overlaps with the absorption spectrum of

the acceptor molecule, the donors may transfer its scattering energy to the acceptor molecule. Thus, the occurring RRS-ET cause the RRS signal decreasing [20,21]. When the donor Fe_3O_4 50nm@AgNP probe is added in the system, the acceptor VB₂ can specifically bind to the binding sites formed on the surface of the Fe_3O_4 50nm@AgNP probe by the MI sites. As the VB₂ concentration in the reaction system increases, the number of vacant specific binding sites in the probe decreases, leading to RRS-ET between the nanoprobe and VB₂, to reduce the RRS signal. Within a certain range, as the VB₂ concentration increases, the decrease in RRS at 380 nm (ΔI) exhibits a linear relationship with VB₂ concentration, thereby a new RRS method for detecting VB₂ was established (Figure 1).

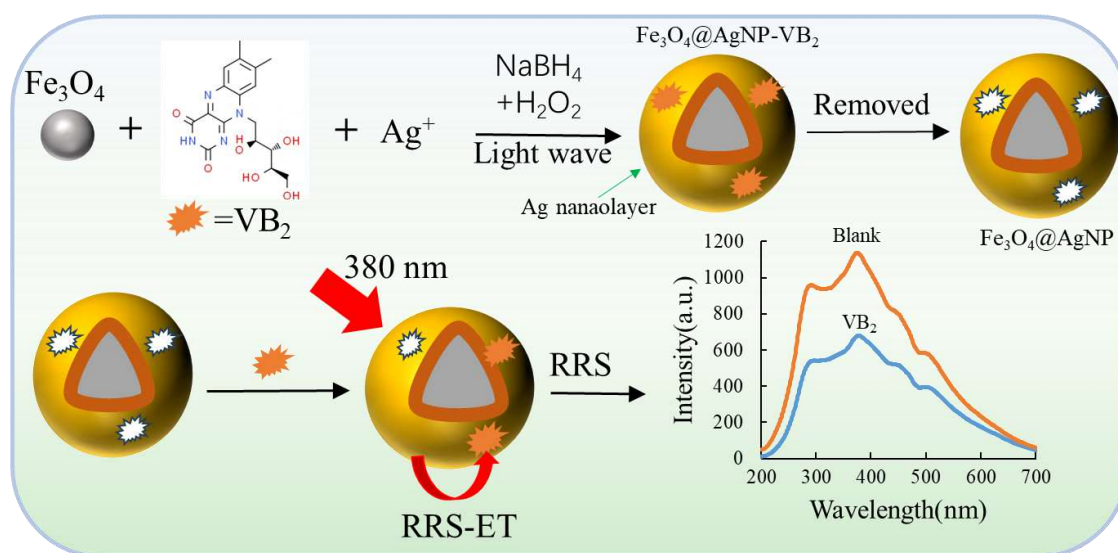


Figure 1 Schematic diagram of RRS-ET detection of VB₂ with Fe_3O_4 @AgNP nanoprobe as donor.

3.2 Characterization of nanomaterials

3.2.1 Molecular spectra

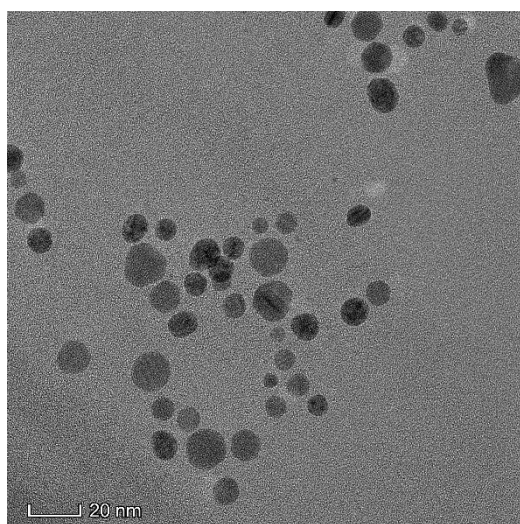
The Fourier transform infrared spectra (Figure S1) indicate the functional groups present in the material. The 50 nm Fe_3O_4 and Fe_3O_4 50nm@AgNP exhibit a broad characteristic absorption peak at 3435 cm^{-1} , which corresponds to the absorption peak of O-H groups in water molecules adsorbed on the surface of the material. Weaker characteristic peaks at 2827 cm^{-1} and 2713 cm^{-1} correspond to the deformation of -OH groups, while peaks at 1593 cm^{-1} and 1350 cm^{-1} are attributed to the characteristic peaks of -COOH groups [29]. Which may be due to the addition of sodium acetate during the preparation of Fe_3O_4 . The wavenumbers at 565 cm^{-1} and 577 cm^{-1} correspond to Fe-O

vibrations. Since silver nanoparticles are generated and adsorbed onto the surface of Fe_3O_4 , the peak intensity of Fe-O in Fe_3O_4 50nm@AgNP is weaker than that in Fe_3O_4 , indicating the formation of Fe_3O_4 50nm@AgNP to some extent. RRS and Abs spectra were recorded for the materials Fe_3O_4 50nm@AgNP, Fe_3O_4 , and AgNP, respectively. As the concentration increased, the both signal values also increased due to nanoparticle number increasing (Figure S2).

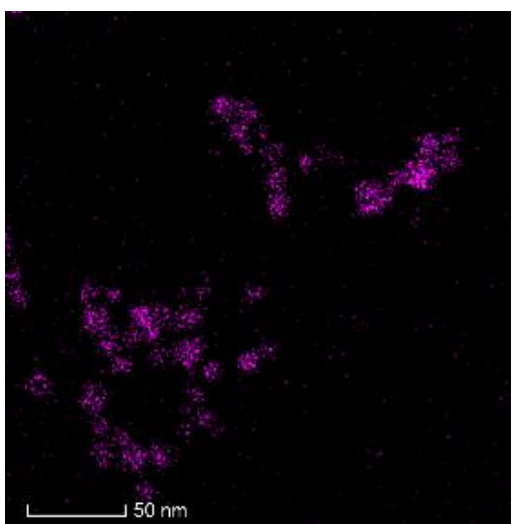
3.2.2 SEM, TEM and EDS analysis

The morphology and size of AgNP, Fe_3O_4 50nm, and Fe_3O_4 50nm@AgNP were characterized using a scanning electron microscope. As shown in TEM Figure. 2A, the average particle size of AgNP is approximately 5 nm, and it contains Ag elements (Figure. 2B). Figures. 2C and 2D show that the size and morphology of Fe_3O_4 50nm@AgNP were changed, with a particle size of approximately 200 nm. From the SEM images of Fe_3O_4 (Figure. 2E) and Fe_3O_4 50nm@AgNP (Figure. 2F), it can be observed that due to the small particle size of AgNP, the morphologies of Fe_3O_4 50nm and Fe_3O_4 50nm@AgNP are similar. From the EDS image of AgNP (Figure. 2G), it can be seen that Ag elements are present, indicating the successful preparation of AgNP. The EDS image of Fe_3O_4 50nm@AgNP (Figure. 2H) shows the presence of O, Fe, Ag, and other elements, which to some extent indicates the successful preparation of Fe_3O_4 50nm@AgNP.

A

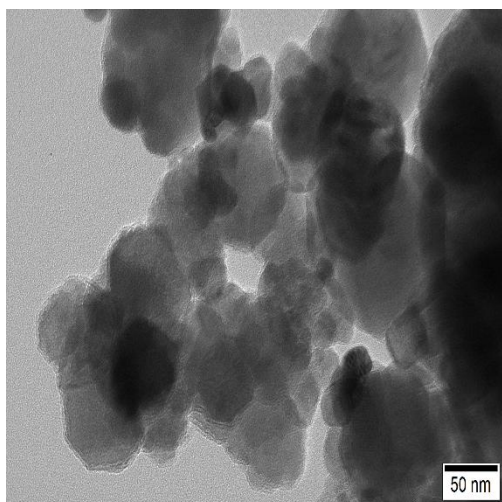


B

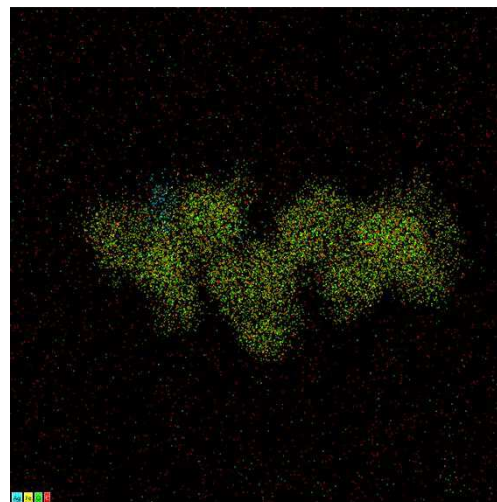


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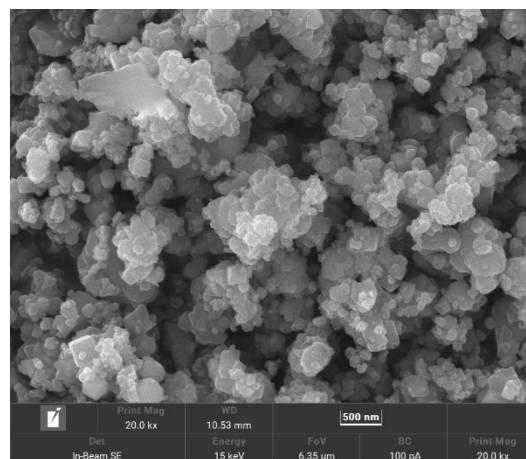
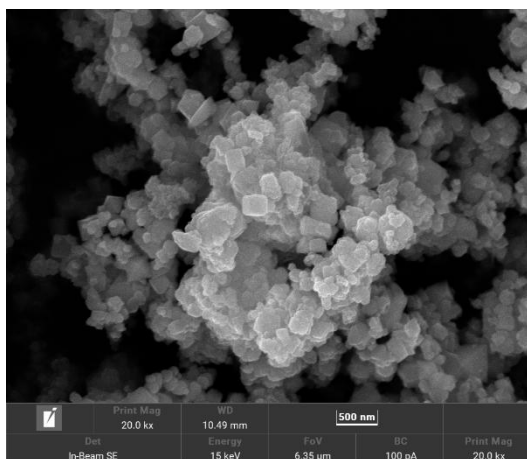
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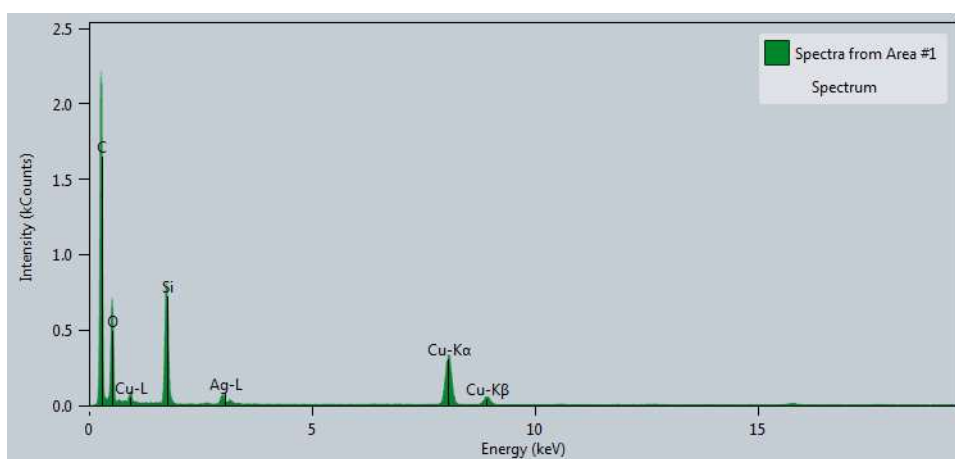
E



F



G



H

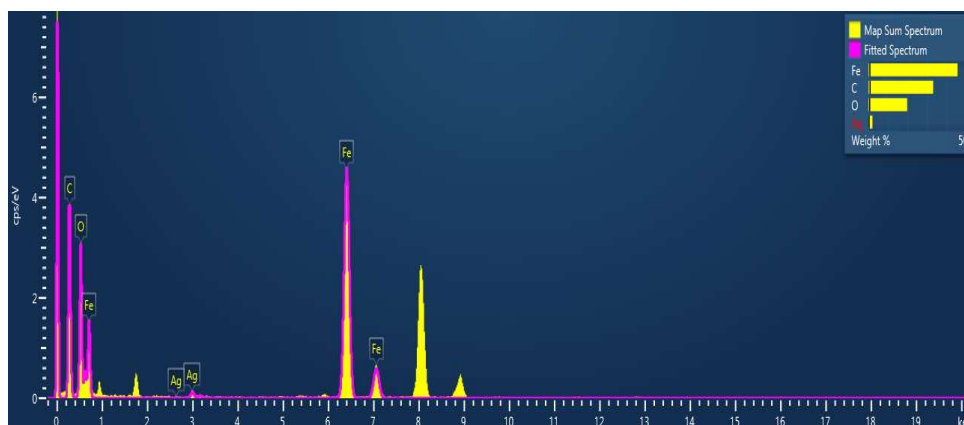


Figure 2 SEM, TEM, and EDS analysis of nanomaterials

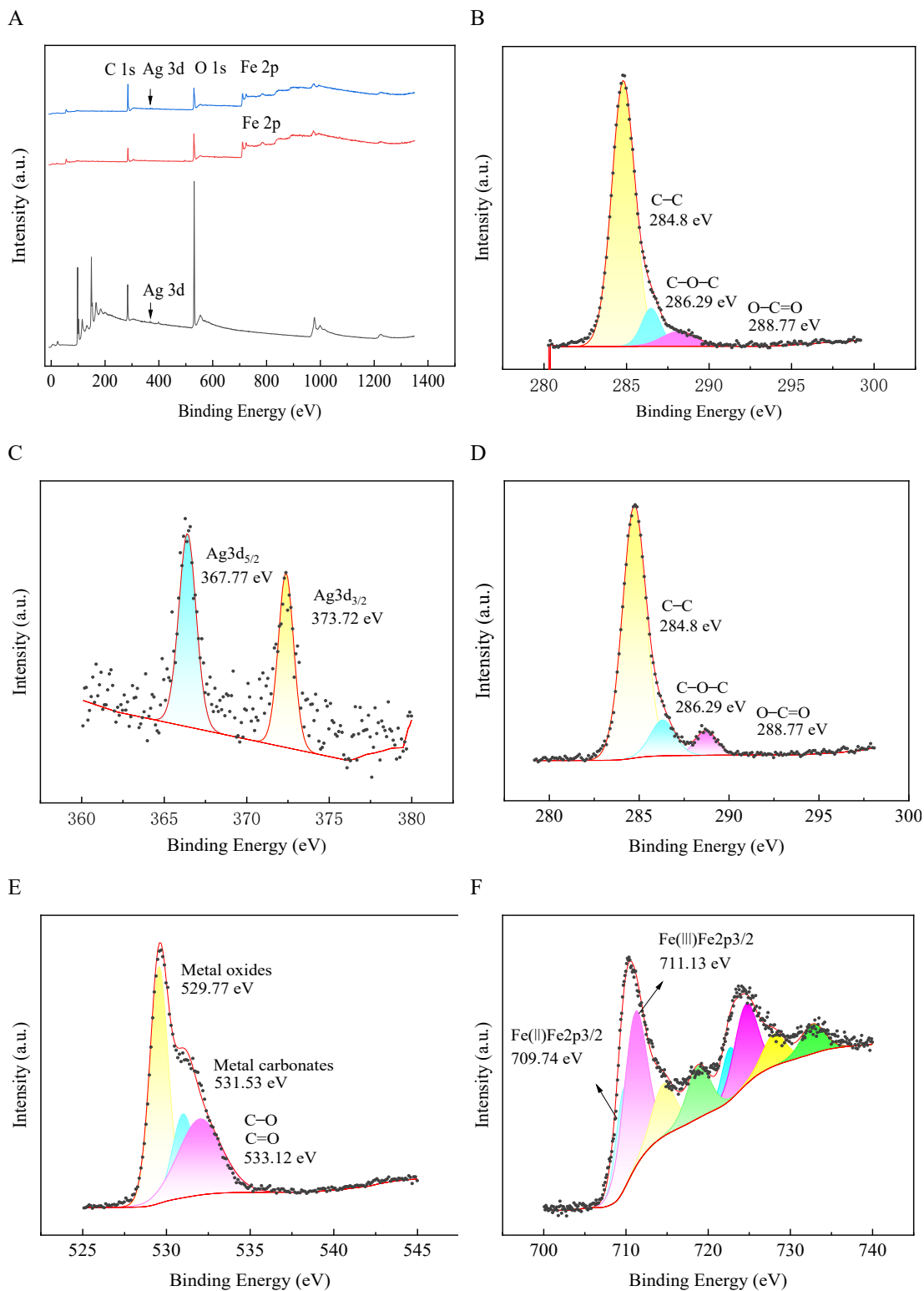
A: TEM of AgNP; B: Mapping of AgNP; C: TEM of Fe₃O₄ 50nm@AgNP; D: Mapping of Fe₃O₄ 50nm@AgNP; E: SEM of Fe₃O₄; F: SEM of Fe₃O₄50nm@AgNP; G: EDS of AgNP; H: EDS of Fe₃O₄ 50nm@AgNP.

3.2.3 X-ray photoelectron spectroscopy (XPS)

To further verify the structure, surface composition, and functional groups of the prepared AgNP, Fe₃O₄, and Fe₃O₄ 50nm@AgNP, we conducted an XPS study. As shown in Figure 3A, the AgNP spectrum exhibits three main peaks at 284.80 eV, 531.14 eV, and 366.95 eV, corresponding to C, O, and Ag elements, respectively. The Fe₃O₄ spectrum exhibits three main peaks at 284.8 eV, 530.4 eV, and 710.93 eV, corresponding to C, O, and Fe elements, respectively. Fe₃O₄ 50nm@AgNP exhibits four main peaks at 284.8 eV, 530.76 eV, 367.77 eV, and 710.53 eV, corresponding to C, O, Ag, and Fe elements, respectively.

The C1s spectrum of AgNP is decomposed into three peaks: C-O-C (286.29 eV), C-C (284.8 eV), and O-C=O (288.77 eV) (Figure 3B). In the high-resolution Ag 3d spectrum (Figure. 3C), the two peaks at 367.77 eV and 373.72 eV correspond to Ag 3d_{5/2} and Ag 3d_{3/2}, respectively. The high-resolution C 1s spectrum of Fe₃O₄ (Figure. 3D) confirms the presence of C-O-C (286.29 eV), C-C (284.8 eV), and O-C=O (288.77 eV) bonds. The O1s spectrum (Figure. 3E) confirms the presence of metal oxides (529.77 eV), metal carbonates (531.53 eV), C=O (533.12 eV), and C-O (533.12 eV) bonds. The Fe 2p spectrum confirms the presence of Fe(II)Fe 2p 3/2 (709.74 eV) and Fe(III)Fe 2p 3/2 (711.13 eV) (Figure 3F). The C1s spectrum of Fe₃O₄ 50nm@AgNP (split into three peaks: C-O-C (286.29 eV), C-C (284.8 eV), and O-C=O (288.77 eV) (Figure 3G). The O1s spectrum (Figure. 3H) confirms the presence of metal oxides (529.77 eV), metal carbonates (531.53 eV), C=O

(533.12 eV), and C-O (533.12 eV) bonds. The Fe 2p spectrum confirms the presence of Fe(II) Fe 2p 3/2 (709.86 eV) and Fe(III) Fe 2p 3/2 (711.30 eV) (Figure. 3I). In the Ag 3 d spectrum (Figure. 3J) shows two peaks at 367.77 eV and 373.72 eV, corresponding to Ag3d5/2 and Ag3d3/2, respectively.



G

H

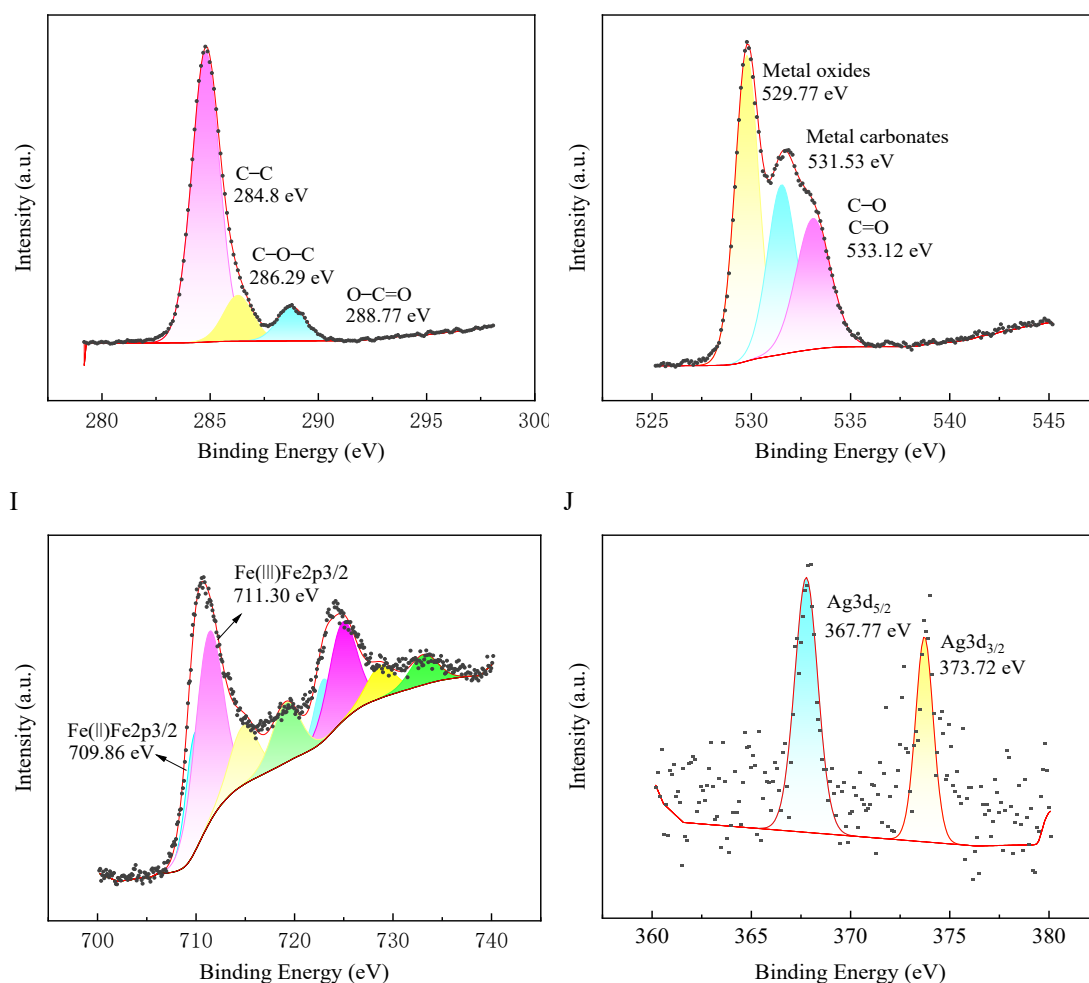


Figure 3 XPS spectra of nanoprobe

A: Complete XPS spectra of AgNP, Fe₃O₄, and Fe₃O₄ 50nm@AgNP; B–C: High-resolution XPS spectra of the C1s, O1s, and Ag3d peaks of AgNP; D–F: High-resolution XPS spectra of the C1s, O1s, and Fe2p peaks of Fe₃O₄. G–J: High-resolution XPS spectra of the C1s, O1s, Ag3d, and Fe2p peaks of Fe₃O₄ 50nm@AgNP.

3.2.4 Stability, Zeta potential, particle size distribution and magnetism

To investigate the stability of the nanoprobe Fe₃O₄ 50nm@AgNP in solution, the simple and sensitive RRS was used (Figure S3). The RSD values of 3.56% indicate that the 25 mg/L Fe₃O₄ 50nm@AgNP sol exhibits good stability in solution. The Zeta potential of the material Fe₃O₄ 50nm@AgNP and Fe₃O₄ was measured, with the following results, the Zeta potential of Fe₃O₄ was -5.09 mV (Figure. S5A), while that of Fe₃O₄ 50nm@AgNP was -10.9 mV (Figure S5B). This indicates that Fe₃O₄ 50nm@AgNP exhibits better stability. Additionally, the difference in potential between the two materials indicates that the Fe₃O₄ 50nm@AgNP nanoprobe preparation was successful. A

particle size analysis of $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ revealed a particle size range of 190–220 nm, due to the loose aggregations. The strength of magnetic properties is a key factor in magnetic separation applications involving magnetic materials. The magnetic properties of Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{AgNP}$ nanomaterials were characterized using a vibrating sample magnetometer (VSM). As shown in Figure S5, the relative saturation magnetization of Fe_3O_4 is 81.56 eum/g, while that of $\text{Fe}_3\text{O}_4@\text{AgNP}$ is 79.45 eum/g. This is because the surface of Fe_3O_4 is coated with AgNP, and an increase in the proportion of non-magnetic materials leads to a decrease in the relative saturation magnetization of the resulting magnetic composite material.

3.3 RRS spectra of the analysis system

Using VB_2 as a template and AgNP as the imprinting precursor, $\text{Fe}_3\text{O}_4@\text{AgNP}$ was prepared for the specific recognition and detection of VB_2 . As shown in Figure. 4, the spectral lines are distinguished by different colors. From the RRS spectrum, RRS peaks are observed at 320 nm and 380 nm, with the RRS peak at 380 nm being the strongest and most sensitive. Therefore, the RRS peak at 380 nm was selected as the basis for detection. The signal intensity at 380 nm decreases gradually with increasing VB_2 concentration, resulting in the RRS-ET effect. The change in RRS signal intensity (ΔI) exhibits a good linear relationship with VB_2 concentration. Additionally, we found that $\text{Fe}_3\text{O}_4@\text{AgNP}$ prepared using Fe_3O_4 with different particle sizes as the substrate can all detect VB_2 , with $\text{Fe}_3\text{O}_4@\text{AgNP}$ prepared using 50 nm Fe_3O_4 as the substrate exhibiting the highest sensitivity for VB_2 detection.

RRS-ET can occur when the RRS spectrum of a nanoprobe donor exhibits spectral overlap with the absorption spectrum of an acceptor species. In this system, the $\text{Fe}_3\text{O}_4@\text{AgNP}$ probe acts as the RRS donor, generating a strong RRS peak at 380 nm (Figure 5a). Upon addition of VB_2 acceptor, the absorption band of the VB_2 (Figure 5d) show considerable overlap with the RRS spectra of $\text{Fe}_3\text{O}_4@\text{AgNP}$ (Figure 5a). Consequently, the donor and acceptor taken place an RRS-ET process, leading to a decrease in the RRS intensity at 380 nm (Figure 5b).

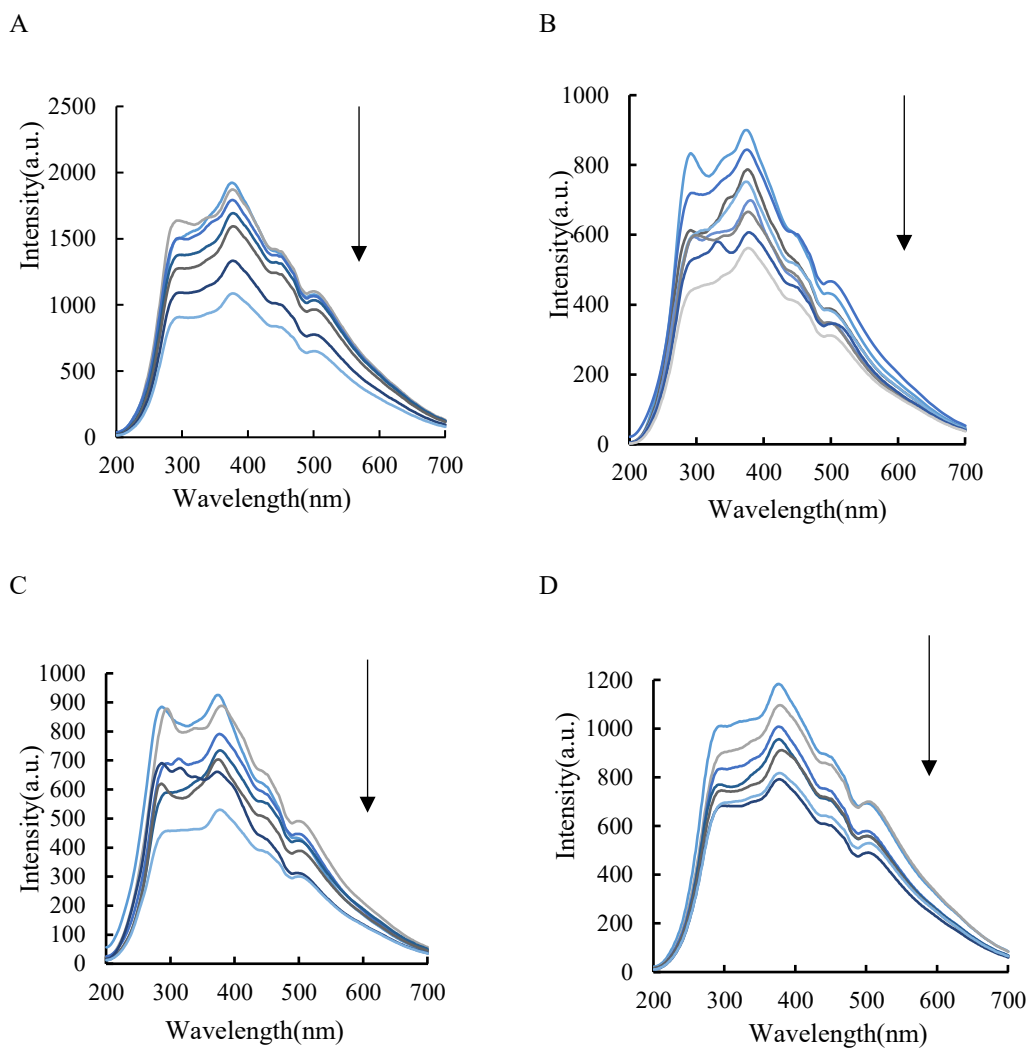


Figure 4 RRS spectra of the $\text{Fe}_3\text{O}_4@\text{AgNP}-\text{VB}_2$ -pH 4.4 buffer solution system

A: 2.5 mg/L $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ - (0, 5, 20, 40, 50, 90, 100, 120 nmol/L) VB_2 ; B: 2.5 mg/L $\text{Fe}_3\text{O}_4_{100\text{nm}}@\text{AgNP}$ - (0, 25, 125, 25, 375, 500, 750 nmol/L) VB_2 ; C: 2.5 mg/L $\text{Fe}_3\text{O}_4_{200\text{nm}}@\text{AgNP}$ - (0, 100, 500, 700, 1000, 1200, 1500 nmol/L) VB_2 ; D: 2.5 mg/L $\text{Fe}_3\text{O}_4_{500\text{nm}}@\text{AgNP}$ - (0, 100, 500, 700, 1000, 1200, 1500 nmol/L) VB_2 .

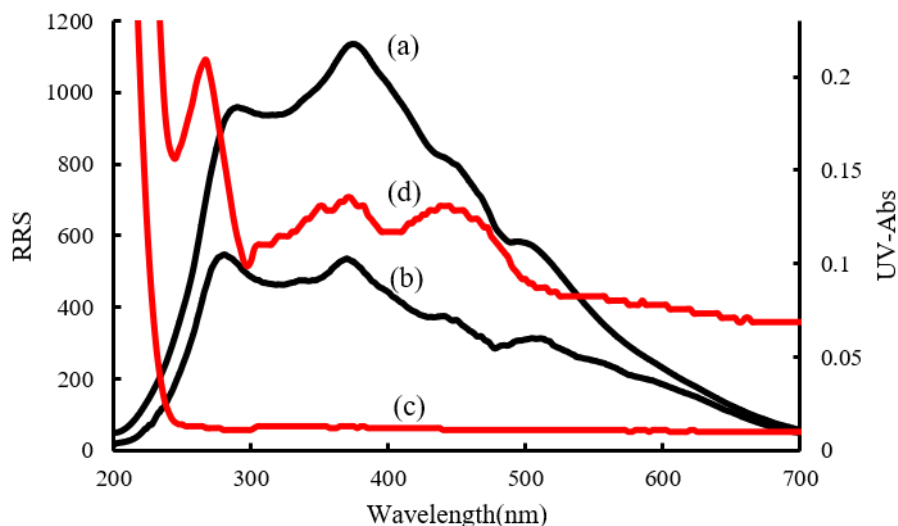


Figure 5 Overlap between RRS and Abs spectra of $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ -VB₂ systems

(a) RRS, 2.5 mg/L $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ + pH 4.4 buffer solution; (b) RRS, (a) + 1.2 $\mu\text{mol/L}$ VB₂; (c) Abs, 2.5 mg/L $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ + pH 4.4 buffer solution; (d) Abs, (c) + 1.2 $\mu\text{mol/L}$ VB₂.

3.4 Condition optimization

3.4.1 Probe preparation conditions.

Elute $\text{Fe}_3\text{O}_4@\text{AgNP}$ five times with 10 mL of eluent to obtain the eluent, its UV-visible absorption spectra were shown in Figure S1. The strongest absorption peak of VB₂ is at 444 nm, and was chosen for evaluating whether VB₂ being eluted or not.

To prepare the most effective nanoprobe, a single variable procedure was used to optimize the preparation conditions for SMIP when VB₂ was 0.04 mmol during the preparation of $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$. Using ΔI as the maximum value as the selection criterion, the influence of factors such as the amount of Fe_3O_4 , functional monomers, and preparation time on ΔI in the $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ analytical system was investigated. The results showed that when the amounts of Fe_3O_4 , AgNO_3 , sodium citrate, H_2O_2 , and NaBH_4 were 35 mg (Figure S7A), 0.02 mmol (Figure. S7B), 0.3 mmol (Figure S7C), 0.6 mmol (Figure S7D), and 0.06 mmol (Figure S7E), respectively, the $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ prepared by reacting at 250°C for 15 min (Figure S7F) under light had the best sensitivity for measuring VB₂.

3.4.1 Analytical conditions

The analysis conditions of the $\text{Fe}_3\text{O}_4_{50\text{nm}}@\text{AgNP}$ system were optimized according to the

experimental procedure. The results showed that when 37.5 mg/L Fe_3O_4 50nm@AgNP was added (Figure S8A) and the pH was 4.4 (Figure S8B), the ΔI 380 of the system was at its maximum. Thus, this analytical conditions were selected for use.

3.5 Work curve

It can be seen from Table 1 that the linear regression equation of the most sensitive system of Fe_3O_4 50nm@AgNP for detecting VB₂ is $\Delta I = 6.15C + 56.2$, with a linear range of 5.0-120 nmol/L and a linear correlation coefficient of $R^2 = 0.972$. The standard deviation (Sd) was obtained by parallel measurement of the blank sample 11 times. According to the calculation formula of the detection limit (LOD), $\text{LOD} = 3.3 \times \text{Sd} / N$ (N is the slope of the working curve), the LOD of RRS can be obtained as 1.7 nmol/L. The non-molecular imprinted polymer of Fe_3O_4 50nm@AgNP_N is least sensitive, due to lack molecular imprinted sites. Compared to some reported method for VB₂ [29-32], this new RRS method is of highly sensitive, selective and simple.

Table 1 RRS-ET analysis features for VB₂.

SMIP	Working curve	Linear range (nmol/L)	Coefficient (R)	LOD (nmol/L)
Fe_3O_4 50nm@AgNP	$\Delta I = 6.15 C + 56.2$	5.0-120	0.986	1.7
Fe_3O_4 100nm@AgNP	$\Delta I = 0.40 C + 33.8$	25-750	0.985	26
Fe_3O_4 200nm@AgNP	$\Delta I = 0.24 C - 1.2$	100-1500	0.990	43
Fe_3O_4 500nm@AgNP	$\Delta I = 0.26 C + 26.9$	100-1500	0.990	39.
Fe_3O_4 50nm@AgNP _N	$\Delta I = 0.13 C + 38.4$	250-750	0.962	100

Table 2 Comparison of some reported methods for VB₂

Method	Principle	Detection range	LOD	Evaluation	References
HPLC*	Diode array detection	—	40 μg/100 g	Accurate, but expensive.	[29]
Electrochemical	Porous silica film	20 nM–7.0	11 nM	Simple, and	[30]

method	modified carbon electrode	μM		good renewability	
	Radio frequency				
Electrochemical	electrochemical	0.004–0.10		sensitive,	[31]
method	transmission of Pd- Cu@NSC/SPCE	μM	7.6 pM	but complex	
	Use the isotope			sensitive,	
LC-MS	dilution method of LC- MS/MS	0.5-50.0 ng/mL	—	but complex.	[32]
	The donor			Highly	
RRS	$\text{Fe}_3\text{O}_4\text{@AgNP}$ probe and acceptor VB_2 taken place ET.	5.0-120 nmol/L	1.7 nmol/L	sensitive, selective and simple.	This method

289 * HPLC-high performance liquid chromatography, MS-mass spectrometry.

290 3.6 Interference experiment

291 The influence of coexisting substances (structurally similar substances, cations and anions) in
 292 the system on the detection of VB_2 was analyzed by the RRS detection method. Taking the RRS
 293 signal value at 380 nm without coexisting substances as the blank I_0 , calculate $\Delta I = I - I_0$. The
 294 experimental results show that the relative error is within $\pm 10\%$. For the detection of coexisting
 295 substances, 1000 times Mn^{2+} , Mg^{2+} , CO_3^{2-} , NO_3^+ , Ni^{2+} , I^- , Cd^{2+} , As^{3+} , VB_{12} , VB_6 and nicotinamide,
 296 900 times Fe^{3+} have no interference on the detection of VB_2 (Table S1). Therefore, this method has
 297 good selectivity. Referring [33], we conducted $\text{Fe}_3\text{O}_4_{50\text{nm}}\text{@AgNP}$ probe and $\text{Fe}_3\text{O}_4_{50\text{nm}}\text{@AgNP}_\text{N}$
 298 recognizing VB_2 and magnet separation experiments. Results indicate that $\text{Fe}_3\text{O}_4_{50\text{nm}}\text{@AgNP}$ probe
 299 can recognize VB_2 , and $\text{Fe}_3\text{O}_4_{50\text{nm}}\text{@AgNP}_\text{N}$ is not recognizing.

300 3.7 Sample analysis

301 The milk samples were purchased from a local supermarket. A 5.0 ml of milk to 15 ml of
 302 acetonitrile was added, then was centrifuged at 3000 rpm for 15 min. Filtered the supernatant using
 303 a 0.22 μm microporous membrane filter, and collected the filtrate as the actual test sample^[28]. The

content of VB₂ in the samples was determined according to the procedure, with each sample tested in parallel five times, and the relative standard deviation (RSD) was calculated. A known amount of VB₂ was added to the samples, and the recovery was calculated. The experimental results showed that the RSD was ranged from 0.23 % to 1.8 %, and the recovery was ranged from 96.34% to 107.85%. The samples were analyzed by high performance liquid chromatography (HPLC)-UV visible detector, the RRS results were agreement with that of HPLC results. This indicate that the RRS method accuracy and reliable.

Table 3 Analytical results of VB₂ in milk samples

Sample	Average (nmol/L)	Added (nmol/L)	Found (nmol/L)	Recovery (%)	RSD (%)	Content (mg/100g)	HPLC (mg/100g)
Milk1	63.59	20	85.16	107.85	1.27	0.190	0.182
Milk 2	57.37	20	76.64	96.34	0.23	0.170	0.166
Milk 3	40.80	20	60.82	100.09	0.70	0.120	0.125
Milk 4	29.85	20	50.88	105.14	0.73	0.090	0.086
Milk 5	80.63	20	100.17	97.69	0.49	0.240	0.248
Milk 6	100.78	20	120.45	98.36	1.8	0.300	0.284

4. Summary

In this paper, a new magnetic molecularly imprinted silver nanoparticle probe (Fe₃O₄_{50nm}@AgNP) was prepared for specific recognition of VB₂ using AgNP surface molecular imprinting technology. When VB₂ specifically binds to the imprinting sites on the probe surface, the RRS-ET effect occurs. As the concentration of VB₂ increases, the RRS signal value decreases, and the both exhibit a linear relationship. Thus, a simple, selective, and sensitive AgNP MI RRS method was established for detecting VB₂, with a detection limit of 1.67 nmol/L. When it was applied to milk analysis, the results were satisfactory with RSD values of 0.23 %-1.8 %, and the recovery of 96.34%-107.85%.

Credit author statement

Dan Li and Yue Luo were equality to the work. Dan Li and Yue Luo: Investigation, Data curation, Writing-original draft. Chongning Li: Methodology, Investigation, Data curation, Software, Funding acquisition. Guiqing Wen: Validation, Data curation, Supervision, Funding acquisition. Aihui Liang: Supervision, Funding acquisition. Zhiliang Jiang: Conceptualization, Writing-review & editing, Supervision, Funding acquisition.

Data availability

The data that has been used is confidential.

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Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

This research did not involve human or animal samples.

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