Preparation of sulfamethazine molecularly imprinted polymer by electron beam irradiation polymerization

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Abstract. Molecularly imprinted polymers (MIPs) of sulfamethazine (SMZ) were prepared initiated by electron beam irradiation in acetonitrile (ACN) with the use of SMZ as template molecule, acrylamide (MAA) as a functional monomer and ethylene glycol dimethacrylate (EGDMA) as a cross-linking agent. The combination of SMZ with MAA and EGDMA was studied by infrared spectroscopy. The adsorption kinetics experiment and selectivity of sulfamethazine imprinted polymers were investigated by ultraviolet spectrometry. The experimental results showed that there was a binding effect among SMZ, MAA and EGDMA. When the molar ratio of SMZ, MAA and EGDMA was 1:4:20, and the irradiation dose was 150 kGy, the adsorption capacity of MIP for sulfamethazine (Q_{MIP}) was the largest (31 mg·g^{-1}). Furthermore, the imprinting factor (F) was 12.91, and the separation factor (α) for SMZ and sulfonamide (SNM) was 1.77.

1. Introduction
Sulfonamides (SAS) is a kind of antibacterial drugs with p-aminobenzensulfonamide structure. The excessive residue of SAS in food and environment will bring a lot of harm, such as allergic reaction, liver damage, renal injury and the formation of urinary stones. Sulfadimidine (SMZ) is one of the most commonly used sulfonamides [1]. It is not easy to be degraded and accumulated in the water environment, which seriously affects water safety [2]. Therefore, how to effectively detect and control sulfamethazine residues in food and water environment has been paid more and more attention. At present, high performance liquid chromatography (HPLC) is the main method for the detection of SAS [3], and liquid chromatography-mass spectrometry (LC-MS) is used for confirmation [4], and non-specific solid phase extraction column is used for pretreatment [5]. Because of its low selectivity, the composition of the sample extract is still very complex, which makes the loss of chromatographic column and detector larger. In mass spectrometry detection, interfering substances will also affect the ionization of the analyte and affect its accuracy. If molecularly imprinted polymers (MIPs) can selectively adsorb SAS, trace SAS in complex samples can be separated, then the detection limit can be reduced, and the accuracy and sensitivity of analysis can be improved.

MIPs is a kind of polymer material which can recognize specific target molecules by molecular imprinting technology. The preparation process generally includes three steps: (1) firstly, select the
appropriate functional monomer to form a reversible complex with the template molecule in the pore forming solvent; (2) adding cross-linking agent and initiator to initiate monomer polymerization under the condition of heating or ultraviolet light, so that the template molecule is embedded in the polymer material; (3) the template molecule is removed from the polymer material with elution. The size, shape and structure of the three-dimensional holes in the materials match the template molecules, so the synthesized MIPs can specifically recognize and bind to the template molecules. In addition to the initiation of radical polymerization by heating or UV irradiation, MIPs can also be prepared by electron beam irradiation [6]. In the former, azodisobutyronitrile (AIBN) is usually used as the initiator of free radical polymerization with long reaction time, strict reaction conditions and poor structure products. But the irradiation method does not need to add initiator and the product is more pure. The radiation energy is large, so the irradiation polymerization reaction can be carried out at room temperature or low temperature. The penetration ability is strong, so the reaction can go deep into the material and the reaction time is short. Additionally, the degree of reaction can be controlled by controlling the irradiation dose, which has a high utilization rate of free radicals. The preparation of SMZ molecularly imprinted polymers by electron beam irradiation has not been reported.

In this experiment, MIPs of SMZ was prepared by electron beam irradiation with SMZ as template molecule, MAA as functional monomer, EGDMA as crosslinking agent and ACN as solvent in our work. The effect of irradiation dose was mainly investigated. MIPs were prepared under the optimal irradiation dose. The adsorption properties of MIPs to SMZ and its analogues SNM were studied and compared. The experimental results are expected to provide a reference for the synthesis of MIPs of SMZ by electron beam irradiation.

2. Experimental

2.1. Materials
Sulfamethazine (SMZ), sulfanilamide (SNM), ethylene glycol dimethacrylate (EGDMA) and methacrylic acid (MAA) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Other reagents were obtained from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). All the reagents used in the experiment were of analytical grade.

2.2. Preparation of sulfamethazine molecularly imprinted polymers
Preparation of MIPs: SMZ (0.2783 g, 1 mmol), MAA (0.3444 g, 4 mmol) and solvent ACN (10 mL) were added into a conical flask under the condition of nitrogen, and the ultrasonic vibration was conducted for 30 min to make them fully react. Four PE bags were prepared and numbered 1, 2, 3 and 4. Take out 3.0 mL of mixed solution and put it into each PE bag together with EGDMA (4 mmol, 0.7928 g). Mix the mixtures thoroughly, vacuum and seal the bags. The mixtures were placed in an electron accelerator (1 MeV, WASK company, USA) and irradiated with 120 kGy, 150 kGy, 170 kGy and 190 kGy doses respectively. The solid materials (MIP1, MIP2, MIP3 and MIP4) obtained after irradiation was crushed and passed through 120 mesh molecular sieve. In Soxhlet extractor, the MIPs were eluted with glacial acetic acid and methanol solution of 1 : 9 (volume ratio) for 3-4 times (each time for 8 h), until no SMZ was detected in the eluent by spectrophotometer. Then wash repeatedly with methanol to neutral to remove acetic acid, and put them into drying oven at 60 ℃ for standby.

Preparation of blank molecularly imprinted polymer (NMIP): The preparation method is the same as above, but without template molecule SMZ.

2.3. Characterization
Ultraviolet-visible spectrum (UV-Vis). In the elution experiment, the concentration of template molecules in the elution solution was determined by a Visible spectrophotometer (7200, Unico, Shanghai, China). In the adsorption experiment, the concentration of template molecules and substrate molecules in the solution was determined by a UV-Vis spectrophotometer (UV 2300, Hitachi, Japan).
Fourier transform infrared spectroscopy (FTIR). The FTIR spectrum of all samples were measured at a FTIR spectrophotometer (Spectrum Two L1600300, Perkin Elmer, USA).

2.4. Adsorption experiment

2.4.1. Static adsorption experiment
Six equal parts of MIPs and six equal parts of NMIPs were weighed in 30.0 mg and placed in the colorimetric tube with stopper, then 0.5 mmol·L⁻¹ SMZ solution in ACN was added. Magnetic stirring was kept at room temperature during the adsorption experiment. Take one part of MIPs and one part of NMIPs solution every one hour, filter, collect filtrate, dilute with ACN for certain times, and then measure the absorbance. The concentration of SMZ after adsorption was calculated according to the standard curve (absorbance-concentration curve) of SMZ. Then the equilibrium adsorption capacity $Q$ and imprinting factor $F$ were calculated by Formula 1 and 2 respectively [7].

$$Q = \frac{(C_0 - C_t) \times V \times M}{m}$$  \hspace{1cm} (1)

$$F = \frac{Q_{\text{MIP}}}{Q_{\text{NMIP}}}$$  \hspace{1cm} (2)

In Formula 1, $Q$ is the adsorption capacity of polymer to substrate (mg·g⁻¹), $C_0$ is the initial concentration of substrate (mmol·L⁻¹), $C_t$ is the equilibrium concentration of substrate after adsorption (mmol·L⁻¹), $V$ is the volume of solution (mL), $M$ is the relative molecular weight of substrate (g·mol⁻¹) and $m$ is the mass of polymer (mg). In Formula 2, $Q_{\text{MIP}}$ and $Q_{\text{NMIP}}$ represent the adsorption capacity (mg·g⁻¹) of MIP and NMIP, respectively.

2.4.2. Specific adsorption experiment
Weigh 30.0 mg MIPs and NMIPs into the colorimetric tube respectively, add 0.5 mmol·L⁻¹ SNM solution in ACN at the same time, incubate in a constant temperature oscillator for 4 h, and then centrifuge to collect the clear solution. All the supernatant was transferred into the colorimetric tube, and the absorbance was measured after being diluted by ACN. The concentration of SNM after adsorption was calculated from the standard curve (absorbance-concentration curve) of SNM. The equilibrium adsorption capacity $Q$ of the polymer is calculated according to Formula 1, and the static partition coefficient $K$ and separation factor $\alpha$ are calculated according to Formula 3 and 4 [8].

$$K = \frac{C_P}{C_S}$$  \hspace{1cm} (3)

$$\alpha = \frac{K_i}{K_j}$$  \hspace{1cm} (4)

In Formula 3, $C_P$ is the concentration of polymer binding substrate (mmol·L⁻¹), and $C_S$ is the equilibrium concentration of substrate in solution (mmol·L⁻¹). In Formula 4, i and j denote template molecules and substrate molecules respectively. When $i = j$, $\alpha = 1.000$.

3. Results and discussion

3.1. Infrared spectrum analysis
Infrared spectroscopy is an effective method to study organic molecules with asymmetric structure and polar functional groups. The changes of polymers structure induced by irradiation can be investigated by infrared spectroscopy. It can be seen from Figure 1 that there are some identical absorption peaks in curve a, b and c. Among them, the stretching vibration peak of O-H is between 3500 cm⁻¹ and 3300 cm⁻¹, the stretching vibration peak of C=O in monomer MAA and crosslinking agent EGDMA is about 1725 cm⁻¹, and the stretching vibration absorption peak of C-O-C in EGDMA is about 1155 cm⁻¹. According to the curves c and d, 3442 cm⁻¹, 3342 cm⁻¹ and 3241 cm⁻¹ were the stretching vibration peaks of -NH₂ and -NH- in SMZ. After polymerization, the absorption of the three peaks became weaker and moved to 3455 cm⁻¹, 3380 cm⁻¹ and 3264 cm⁻¹ respectively. 1639 cm⁻¹ and 1596 cm⁻¹ were the characteristic absorption peaks of pyrimidine ring in SMZ. After polymerization, they moved to 1731 cm⁻¹ and 1638 cm⁻¹. 1302 cm⁻¹ and 1140 cm⁻¹ were the characteristic absorption peaks of O=S=O in SMZ. After polymerization, they moved to 1390 cm⁻¹ and 1155 cm⁻¹. At the same time, curve c
retains the fingerprint absorption peak of SMZ in curve d. After elution, the fingerprint absorption peak of SMZ in curve c and absorption at 3380 cm\(^{-1}\) and 3264 cm\(^{-1}\) gradually disappeared, which was also reflected in curve a. At the same time, the stretching vibration peak of C=O moved from 1731 cm\(^{-1}\) to 1725 cm\(^{-1}\). To sum up, we can infer that the template molecule SMZ may be hydrogen bonded with -COOH in MAA or C=O in crosslinking agent EGDMA through amino group, imino group, pyrimidine ring and sulfonic group. In addition, SMZ imprinted in the polymer can be removed by acetic acid and methanol washing. The results showed that SMZ could be imprinted into the polymer and eluted successfully.

![Figure 1. Infrared spectra of SMZ and polymers](image)

**3.2. Effect of irradiation dose on adsorption capacity**
According to the method of 2.4.1, the adsorption capacity of MIP1-MIP4 for SMZ was determined and calculated. The results are listed in Table 1. It can be seen from Table 1 that when the irradiation dose is 150 kGy, the adsorption capacity of the polymer for SMZ is the largest. In the process of irradiation initiated polymerization, the properties of MIPs prepared mainly depend on the irradiation dose. When the irradiation dose is too low or too high, it is not conducive to the adsorption of SMZ. When the irradiation dose is low, the concentration of free radicals in the system is low, and the degree of cross-linking is low. The particle size of MIP solid material is too small, which is not conducive to adsorption. When the irradiation dose is too high, excessive free radicals will lead to the rapid increase of polymerization degree and viscosity of the system, resulting in the rapid increase of the particle size of the solid materials. In this process, the site of action may be embedded in the material, which is not conducive to the elution and binding of target molecules [6]. Therefore, the irradiation dose of 150 kGy was selected to initiate the preparation of MIPs.

| Irradiation dose (kGy) | MIP    | \(Q_{\text{MIP}}\) (mmol·g\(^{-1}\)) |
|-----------------------|--------|----------------------------------|
| 120                   | MIP1   | 0.00965                          |
| 150                   | MIP2   | 0.02501                          |
| 170                   | MIP3   | 0.01946                          |
| 190                   | MIP4   | 0.01762                          |

**3.3. Comparison of adsorption equilibrium between MIP4 and NMIP for SMZ**
In order to compare the adsorption properties of MIP4 and NMIP for SMZ, adsorption experiments with time as variable were carried out within 6 h. The results are shown in Fig. 2. It can be seen from Fig. 2 that the adsorption rate of MIP4 is higher in the first four hours, and the adsorption capacity changes greatly with the increase of time. The adsorption rate changes little after four hours, and the adsorption tends to be steady. At the beginning, the high adsorption rate was due to the existence of a large number of holes left after the elution of SMZ in MIP4. However, with the passage of time, the holes in the MIP were filled with template molecules, so the adsorption rate slowed down and the adsorption reached equilibrium after 4 hours. There is no SMZ template molecule in NMIP, and there is no specific action site after elution, so there is no specific adsorption for SMZ, and the adsorption is mainly surface adsorption. The adsorption equilibrium of NMIP was rapidly established after 1 h, but the adsorption capacity was small and the adsorption performance was poor. At the same time, from Figure 2 and Formula 2, we can see that the equilibrium adsorption capacity of MIP4 \( (Q_{MIP}) \) is 31 mg·g\(^{-1}\), the equilibrium adsorption capacity of NMIP \( (Q_{NMIP}) \) is 2.4 mg·g\(^{-1}\) and the imprinting factor \( (F) \) is 12.91. This indicates that MIP4 and NMIP have obvious differences in the adsorption of SMZ.

### 3.4 Comparison of adsorption of MIP4 and NMIP for different substrates

The adsorption of template molecular structure analogue SNM by MIP4 and NMIP is shown in Table 2. It can be seen from table 2 that the adsorption properties of NMIP for SMZ and SNM have no significant difference (2.4 mg·g\(^{-1}\) and 3.5 mg·g\(^{-1}\) respectively), while the adsorption properties of MIP for SMZ and SNM are significantly different (31.0 mg·g\(^{-1}\) and 7.0 mg·g\(^{-1}\) respectively). The adsorption capacity of MIP4 for SMZ was significantly greater than that for SNM, which indicated that MIP4 had specific adsorption for SMZ. Furthermore, according to Formula 3 and 4, it can be concluded that \( K_i \) is 2.28, \( K_j \) is 1.29 and \( \alpha \) is 1.77, indicating that MIP4 has good adsorption and specific recognition.

### Table 2. Comparison of adsorption of MIP4 and NMIP for SMZ and SNM

| Substrate | \( C_0 \) (mmol·L\(^{-1}\)) | \( C_t \) (mmol·L\(^{-1}\)) | \( Q_{MIP} \) (mg·g\(^{-1}\)) | \( Q_{NMIP} \) (mg·g\(^{-1}\)) |
|-----------|----------------|----------------|----------------|----------------|
| SMZ       | 0.50           | 0.219          | 31.0           | 2.4            |
| SNM       | 0.50           | 0.388          | 7.0            | 3.5            |

### 4. Conclusion

In this experiment, SMZ molecularly imprinted polymer was successfully prepared by electron beam irradiation. It has the advantages of short reaction time, low temperature and better purity. The results showed that the maximum specific adsorption capacity was obtained when the irradiation dose was 150 kGy and the ratio of template molecule to functional monomer and crosslinker was \( 1 : 4 : 20 \). The adsorption capacity of MIP for SMZ \( (Q_{MIP}) \) was 31 mg·g\(^{-1}\) and the imprinting factor \( (F) \) was...
12.91. The separation factor (α) for SMZ and SNM was 1.77. This experiment has a certain guiding significance for the preparation of MIPs.

Acknowledgments
This work was supported by the guiding project of science and technology from Hubei Provincial Education Department (B2019168), the doctoral initiation fund of Hubei University of science and technology (BK201801), and the key teaching and research project of Hubei University of science and technology (2018-XA-010).

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