Optimization of Preparation Method of Molecularly Imprinted Microspheres by Uniform Design

Qiang Zhou¹,², Hong Zhang¹,² and Yanhong Wang¹,²*

¹ Institute of Applied Ecology, Chinese Academy of Sciences, No. 72 Wenhua Road, Shenyang 110016, China
² Ministry of Agriculture Laboratory of Risk Assessment of Environment Factors for Quality and Safety of Agro-Products, Shenyang 110016, China
Email: wangyh@iae.ac.cn

Abstract. This work presented a simple and effective method for the preparation of molecularly imprinted microspheres (MIMPs) through multi-step swelling polymerization. To prepare carbofuran MIMP with a uniform size, the experiment of optimizing the dosage of reagents which used during synthesis based on uniform design (UD) method was performed. Six kinds of factors, namely methacrylic acid (MAA, functional monomer), ethylene glycol dimethacrylate (EGDMA, cross linker), polyvinyl alcohol (PVA, dispersant), dibutyl phthalate (DBP, sweller), polymerization temperature and polymerization speed were taken into account based on UD to perform the optimization experiment. The micromorphology of MIMP was characterized by scanning electron microscopy (SEM) and it showed that the particle sizes were between 6-8 μm. The results indicated that MIMP possessed good selectivity and specificity for carbofuran, which was an excellent material for adsorption and testing of carbofuran residues in food and environment.

1. Introduction
Carbofuran is a broad-spectrum of carbamate insecticidal and nematode killing agents with contact and stomach toxicity [1]. By combining and inhibiting the activity of acetylcholinesterase in vivo, it causes the accumulation of acetylcholine in tissues. Because the combination is irreversible, the carbofuran residue in soil is very long and the migration in soil is obvious, as well as it can be absorbed by the roots of plants. Carbofuran is defined as a highly toxic pesticide according to the standard of pesticide toxicity classification [2]. The problem of carbofuran residue still remains quite severe and has become an important problem affecting the quality and safety of agricultural products along with people's health. Hence there is badly in need of a reliable, convenient and applicable method for rapid detection of carbofuran in food and environment.

Molecularly imprinted polymers (MIPs) are stable synthetic polymers possessing selective molecular recognition sites, which are obtained in the presence of template molecules by using a large excess of cross linker and monomers. Once the template molecules are removed, specific sites are left behind which can selectively rebind the template molecules and structurally related analytes. Some of these polymers have selectivity and affinity constants comparable with those of naturally occurring recognition systems, such as monoclonal antibodies or receptors. They also have other inherent advantage including high mechanical, high selective recognition, chemical stability and low cost of synthesis [3]. In general, MIPs as micro-sized materials have a better performance for target analytes...
from the complex matrix and corresponding isomer and it is perfect to detect the pesticide micro-residues in fruits and vegetables.

Recently we have proposed a facile strategy for the specific recognition of carbofuran based on the fluorescence quenching of quantum dots labelled MIMPs [4]. In order to make the synthesized carbofuran MIMPs in according with flow cytometry, that is, the particle size is below 38 μm and unique, UD method was chosen to optimize the synthetic conditions of carbofuran MIMPs. In recent years, researchers have developed various experiment design methods, such as whole experiment [5], orthogonal design [6], single factor experiment [7] and uniform design [8], etc. The orthogonal experiment has the characteristics of uniform dispersion, neat and comparable, and the experimental number is the square of the level number. As for whole experiment, in order to obtain the comprehensive test information, all combinations of selected factors on the test level of the full implementation should be implemented more than once. It is generally suitable for investigating the factors and the level of the number of conditions is not too much, mainly for single factor and double factor experiments. Generally, whole experiment and orthogonal design are not suitable for multi-levels and multi-factor experiment, because the experiment number is the square of level number at least. Single factor experiment is more often used as the preparation experiment which provides a reasonable data range for the orthogonal experiment. However, uniform design (UD) is a combination of number theory and multivariate statistics and developed based on orthogonal design. The test site has the characteristics of uniform dispersion, and the experimental number is equal to the level number. Liu et al. used UD combined with fixed-ratio ray design to assess the combined toxicities of multi-component mixtures [9]. Li et al. combined UD and support vector machine to assess probabilistic tunnel stability, a hybrid method for reliability analysis of tunnels was developed [10]. Taking all the above into account, UD applies to the design of multi-level and multi-factor experiment.

In this work, the UD method was used to optimize of the uniformity and sphericity of MIMPs. SEM, Fourier transform infrared spectrometer (FTIR) and high performance liquid chromatography (HPLC) were applied to the characterization of MIMPs. The MIMPs have good adsorption for carbofuran and can be further used for the quantitative analysis of carbofuran.

2. Experimental

2.1. Reagents and Chemicals
All the agents were analytical grade. Carbofuran was purchased from Huayang Chemical Reagent Co., Ltd (Shandong, China). Sodium dodecyl sulfate (SDS) was obtained from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). dibutyl phthalate (DBP), Azobisisobutyronitrile (AIBN) and Polyvinyl alcohol (PVA) were purchased from Tianjin Bodi Chemical Reagent Co., Ltd (Tianjin, China). Toluene was purchased from Zibo Fengcang chemical Co., (Shandong, China). Methacrylic acid (MAA) was from Alfa Aesar Co., (MA, USA). Ethylene glycol dimethacrylate (EGDMA) was from Acros Co., (New Jersey, USA). Doubly deionized water (DDW, 18.2 MΩ cm$^{-1}$) from the Milli-Q system (Billerica, MA, USA) was used to prepare all aqueous solutions.

2.2. Apparatus
The morphology of the MIMPs was observed with a SEM (FEI quanta 250, Netherlands). The FTIR spectra of the QDs-MIP were detected with a FTIR spectrometer (Nicolet, Madison, WI, USA). The adsorption performance of the MIMPs and non-molecularly imprinted microspheres (NIMPs) were examined by HPLC (Waters 2695, USA).

2.3. Preparation of Carbofuran MIMPs and NIMPs
The synthesis of carbofuran MIMPs was performed by a multi-step swelling polymerization method. Firstly, sodium deodecyl sulfate (SDS, 0.02 g), DBP, polystyrene microsphere (Ps, 0.042 g) and DDW (10 mL) were added into a conical flask for ultrasonic for 10 min. Then the reaction system was stirred...
at 150 rpm at room temperature for 15 h. Secondly, AIBN (0.1642 g), toluene (2.5 mL), PVA solution with a mass fraction of 4.8 %, DDW (12.5 mL) were added and stirred at 150 rpm at room temperature for 2h. Thirdly, carbofuran (1 mmol), MAA, PVA (mass fraction of 4.8 %), DDW (12.5 mL) and EGDMA were added sequentially into the mixture and continuously stirred at 150 rpm for 0.5 h. Finally, the mixture was transferred to a three flask bottle to polymerize under a nitrogen environment at a certain temperature for 20 h. The mixture was then redispersed into methanol, and the above procedure was repeated three times in methanol, twice in water and twice in tetrahydrofuran. The template molecules were removed by Soxhlet extraction with 200 mL of methanol and acetic acid (v:v = 9:1) until no carbofuran was detected. After drying in vacuum at room temperature, carbofuran MIMPs were obtained. For comparison study, non-molecularly imprinted polymers (NIPs) were also synthesized without adding carbofuran and used as blank control material.

2.4. Adsorptions Capacity of MIMPs

To examine the adsorption kinetics of MIMPs, 10.0 mg of MIMPs and NIMPs were added into 10 mL of 20 μg.L⁻¹ carbofuran solutions, respectively. The concentration of carbofuran was detected by HPLC at 10 min, 20 min, 40 min, 60 min, 80 min, 100 min, 120 min, 140 min, 160 min and 180 min.

2.5. Factors and Levels of Uniform Design

According to a series of literature research [11] and the summarization of the influence of the agents’ amount for the size distribution and uniform of the MIMPs, the amount of EGDMA, DBP, MAA, PVA, along with the polymerization temperature (T) and polymerization stirring speed (S) were taken into account in this work, as shown in table 1. The relative standard deviation of particle size was taken as the evaluation index.

| Factors | Levels       |
|---------|--------------|
| EGDMA   | 1.98 1.98    |
|         | 2.16 2.35    |
|         | 2.5 2.5      |
|         | 2.64 2.82    |
|         | 3.0 3.2      |
|         | 3.39         |
| DBP     | 0.12 0.12    |
|         | 0.18 0.21    |
|         | 0.24 0.24    |
|         | 0.27 0.3     |
|         | 0.3 0.33     |
|         | 0.33 0.36    |
| MAA     | 2.5 3.0      |
|         | 3.5 4.0      |
|         | 4.0 4.5      |
|         | 5.0 5.5      |
|         | 6.0 6.5      |
|         | 7.0 7.5      |
| PVA     | 8.5 8.8      |
|         | 9.1 9.4      |
|         | 9.7 10.0     |
|         | 10.0 10.3    |
|         | 10.6 10.9    |
|         | 11.2 11.5    |
| T       | 57 59        |
|         | 61 62        |
|         | 63 64        |
|         | 65 67        |
|         | 69 71        |
|         | 73           |
| S       | 250 250      |
|         | 260 260      |
|         | 270 270      |
|         | 280 290      |
|         | 290 290      |
|         | 300 300      |

3. Results and Discussion

3.1. Characterization of MIMPs

The purpose of this study was to prepare carbofuran MIMPs with uniform particle size, so the observation of the particle size was essential. The morphology of the synthesized microspheres was revealed by SEM as shown in figure 1. As we can see from the figure, eleven groups experiments of the uniform designed. Meanwhile the U4, U6, U9 sets of experiments had the uniform particle size compared with the other experiments, relatively. It was preliminarily judged that the above three synthesized microspheres have uniform particle size. The sixth experiment microspheres showed a rather narrow diameter distribution with lower than 3.00 % disperse coefficient. Another indicator output showed in table 2 indicated that the U6 had the largest output. Combine the above two index, it illustrated that U6 was the optimum experimental conditions in the synthesis of carbofuran QDs@MIPs, i.e., the usage of EGDMA was 3.39 mL, DPB was 0.21 mL, MAA was 5.5 mmol, 4.8 % PVA was 9.1 mL in other two steps, polymerization temperature was 71 ℃, polymerization speed was 270 rpm.
Figure 1. SEM images of 11 groups of experiments of uniform design from U1 to U11.

In the case of knowing the best level section, the corresponding experimental conditions of the optimum point in the uniform design are very close to the optimum conditions of the overall experiment because of the level of uniform design is more, the level interval is smaller, and the test points are evenly distributed. In rapid analysis, the optimum condition of the uniform design can be taken as the optical scheme.

3.2. Verification and Characterization of Optimized Conditions

The optical experiment was repeated three times and the SEM images of the amplified microspheres were shown in figure 2. The relatively standard deviations [12] were 2.85%, 2.96%, 2.73%, respectively. It indicated that the method had a good repeatability. Otherwise, the particles which have uniformly distributed diameters had regularly spherical and porous surface.

Figure 2. SEM images of the amplified QDs@MIPs microspheres.

The rebindin experiments for MIPs were conducted in water to verify the adsorption property for carbofuran. On the one hand, for the same concentration of solution, the adsorption rate decreased gradually with the increase of time, and reached the adsorption equilibrium in 100 minutes (figure 3).

On the other hand, the maximum adsorption capacity of MIMPs is about 50 μmol·g⁻¹, and the maximum adsorption capacity of NIMPs is about 35 μmol·g⁻¹ which indicated that the adsorption capacity of MIMPs grams of carbofuran molecular was stronger. Both the adsorption amount of MIMPs and NIMPs increased gradually with the time increasing, but obviously the adsorption capacity of MIMPs was much more than that of NIMPs.
Figure 3. The adsorption kinetics curve of carbofuran MIMPs and NIMPs.

FTIR spectroscopy was used to further characterization MIMPs and NIMPs. As shown in figure 4, the infrared spectrum of MIMPs and NIMPs were similar, but this was not surprising. Both MIMPs and NIMPs were composed of functional monomer and crosslinker especially when the template was removed from MIMPs. A relatively broad peak near 3440 cm\(^{-1}\) was the characteristic peak of O-H of carboxylic group, the peak near 1740 cm\(^{-1}\) was the characteristic peak of C=O, both of the two peaks indicated the existence of carboxylic group in MIMPs. The characteristic peaks of C=C around 1640 cm\(^{-1}\) are not obvious, indicating that the residue of functional monomers was very little, and most of them had reacted with the crosslinking agent.

Figure 4. The FTIR spectra of carbofuran MIMPs and NIMPs.

4. Conclusions

In summary, based on the uniform design experiments and the corresponding statistical analysis, the optimization of the synthetic conditions of carbofuran MIMPs was performed in this paper. The resultant microspheres had uniform particle size and showed good adsorption properties for the template molecule. Dynamic adsorption test showed that it could reach equilibrium within 100 min. The results indicated that MIMP possessed good selectivity and specificity for carbofuran, which was an excellent material for adsorption and testing of carbofuran residues in food and environment.

Acknowledgement

We gratefully appreciate financial support from the Scientific Instrument Developing Project of the Chinese Academy of Sciences (YJKYYQ20180068), the National Key Basic Research Development Project Program (16YFD0401201), the Project Program of Shouguang Facility Agricultural Research Center of IAE CAS (2018SG-Y-07) and the CAS Scholarship. The authors wish to express their gratitude to the anonymous reviewers for the stimulating suggestions and discussions.
References

[1] Sun X, Zhu Y and Wang X 2012 Food Control 28 184
[2] Samphao A, Suebsanoh P, Wonga Y, Pekec B, Jitchareon J and Kalcher K 2013 Int. J. Electrochem. Sci. 8 3254
[3] Kubo T and Otsuka K 2016 Trends Anal. Chem. 81 102
[4] Zhou Q, Liu C, Zhang H, Zhao C and Wang Y 2017 Anal. Sci. 33 957
[5] Baker T B, Smith S S, Bolt D M, Loh W Y, Mermelstein R, Fiore M C, Piper M E and Collins L M 2017 Behavior Therapy 48 567
[6] Liao H Y, Gao H X, Xu B and Liang Z W 2017 Sep. Purif. Technol. 183 117
[7] Sun Y, Xiao S L, Feng L, Liang J Y and Tian Y Y 2014 Forest Engineering 30 50
[8] Wang Y H, Mu J L and Wang J 2011 Frontiers of Agriculture in China 5 407
[9] Liu S S, Xiao Q F, Zhang J and Yu M 2016 Science Bulletin 61 52
[10] Li X, Li X B and Su Y H 2016 Structural Safety 61 22
[11] Ncube S, Kunene P, Tavengwa N T, Tutu H, Richards H, Cukrowska E and Chimuka L 2017 J. Environ. Manage. 199 192
[12] Wang J J, Han Y J, Li J and Wei J 2017 Sep. Purif. Technol. 177 62