Preparation of Fe_3O_4 Magnetic Surface Imprinted Microspheres and the Ethyl Acetate Extract Flavonoids Raspberry Concentration of Active Ingredient Applied

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Abstract.the study is used by the co-precipitation method to make some uniform particle size and have good Fe_3O_4 magnetic nanoparticles and their surface modified with oleic acid ; oleic acid as the carrier of Fe_3O_4, quercetin as template molecule, prepared by the microwave assisted molecular imprinted polymer magnetic nanospheres; In raspberry ethyl acetate extract fingerprints for the assessment index, with orthogonal design best preparation; Characterization of equilibrium adsorption constant Kd and maximum adsorption capacity Qmax by Scatchard model.The results show that: This study explores the preparation of MIPs polymerization time by ten times, prepared by the Fe_3O_4 nanometer level, greatly increase the MIPs of the specific surface area, thereby increase the amount of adsorption (K_d = 0.7322mg / L, Q_{max} = 18.92μmol / g).Successfully extract raspberry flavonoids active ingredients from ethyl acetate which can be used for rapid and large parts of ethyl acetate enrichment raspberry flavonoids.

1. Introduction

Raspberry, as rosaceae plants in east China raspberry Rubus chingii Hu immature fruit, exists in many anti-aging prescriptions. Research on the group of rosaceae plants in eastern China raspberry Rubus chingii Hu, prevention and treatment of senile dementia study of active ingredients: Improve the material basis of raspberry deficiency animal learning and memory function for the ethyl acetate extract raspberry flavonoids[1、2、3].As is known, the active ingredient is a group of traditional Chinese medicine through a multi-channel, multi-channel, multi-level, multi-target function together. Each active ingredient must be compatible combination required to play the best results, different components of different targets, if one of the components is adjusted, the effect is also changed. Therefore, the establishment of a large, rapid separation of flavonoids from raspberry, without affecting

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the other components of the composition is a prerequisite for further research of effective component compatibility and mechanism.

However, it is difficult to achieve separation from the traditional Chinese medicine in a class of active ingredients without affecting the other components coexist from the conventional component separation method. Compared with the traditional separation or analysis of media, the prominent characteristics MIPs is to be separated having specific selectivity, while MIPs have better physical and chemical stability. The main method of preparation of molecularly imprinted polymer emulsion polymerization, suspension polymerization and surface polymerization, polymer film, and so on. Imprinted polymer obtained by these methods, although the target molecule selectively adsorbed, however, it exists the following disadvantages: Polymers require grinding and screening, time-consuming; particle size range or get wide, part of the recognition site is enclosed within the polymer, so that the removal of the template molecule is more difficult; not suitable for industrial enrichment and separation of active ingredients of traditional Chinese medicine.

Magnetic Imprinted Polymer Microspheres small particle size, achieve nanoscale , large surface area, adsorption capacity, can handle a large number of samples[4]. By an applied magnetic field, the MIPs adsorption target molecule can be easily separated from the mother liquor. Imprinted polymer magnetic composite microspheres to overcome the weaknesses of the other MIPs, can meet the necessary conditions for the separation of Chinese medicines, it is an important direction of the ideal material separation and enrichment of reactive component, but also the imprinting technology.

In this study, quercetin molecule as a template synthesized Fe_3O_4 magnetic nanoparticles surface imprinted polymer, and successfully extracted from raspberries ethyl acetate was separated flavonoids active ingredient. After the ethyl acetate extract raspberry extract isolated flavonoids, other components remained unchanged. The synthesis reaction under microwave irradiation, the reaction time is shortened ten times.

2. Reagents and Materials

Sartorius BS214 - D balance (the Sartorius instrument co. Ltd.)；Microwave apparatus (Sineo microwave chemical technology co. Ltd.)；KQ3200 ultrasonic cleaning machine (Kunshan ultrasonic instrument factory Kunshan)；UV-VIS spectrophotometer (UV-1750 SHIMADZU); Agilent 1260 high performance liquid chromatograph (Agilent technologies co. Ltd.); Automatic triple distilled water machine (Shanghai rongsheng biochemical instruments co. Ltd.)；Soxhlet extractor (Nanchang University Glass Instrument Factory)

4 - vinyl pyridine, ethylene glycol dimethyl acrylate, azodiisobutyronitrile and silane coupling agent KH-570 are purchased from Aladdin reagent co.Ltd; azodiisobutyronitrile are purchased from Recovery of Tianjin institute of fine chemicals; $FeSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$ are purchased from Tianjin Guangfu Fine Chemical Research Institute; Hydrazine hydrate is purchased from Sinopharm Chemical Reagent Co., Ltd; Acetonitrile and methanol are chromatographic purity, other reagents are analytical purity; kaempferol and quercetin as reference substance are purified by ourselves(99.11%).

3. Experiments

3.1Preparation of Fe3O4 magnetic nanoparticles and the surface of oleic acid

Take $FeSO_4 \cdot 7H_2O$ of about 4g was dissolved in 120mL triple-distilled water, $FeCl_3 \cdot 6H_2O$ about 10g dissolved in 240mL triple-distilled water. Under nitrogen, to a solution of $FeCl_3 \cdot 6H_2O$ was added with stirring three-necked flask, speed is 600 revolutions / minute, after 10mL of hydrazine hydrate was added dropwise, followed then slowly added to the solution of $FeSO_4 \cdot 7H_2O$, 70mL ammonia, sealed, reaction at room temperature 2h, after 2h placed in a microwave extraction apparatus, under nitrogen, the condition is that microwave power is 200W and temperature is 80 ℃ , closed reaction 0.5h; after the completion of the reaction, 5.7g of oleic acid was added dropwise ,under the condition that microwave power is 200W and temperature is 80 ℃ , closed the reaction 2h; completion of the reaction and then adding 70mL toluene, speed to 300 r / min, microwave power 200W, at a temperature of 80 ℃ conditions, closed reaction 0.5h; after the reaction was transferred to a separatory funnel and the toluene layer was collected, no washed several times with water, ethanol, placed in an oven at 80 ℃ vacuum dried, milled standby, to obtain a surface of oleic Fe_3O_4 magnetic nanoparticles.

3.2Preparation of magnetic Fe3O4 surface imprinting microspheres and process optimization

This study designed a orthogonal experiment to investigate Fe_3O_4 magnetic surface imprinting microsphere preparation relationship among the factors. In accordance with table 1 take appropriate amount of quercetin, join 12 mL of acetone ultrasonic dissolving, filter, filter paper in 3.1 the oil acidification Fe_3O_4magnetic nanoparticles 0.5 g, according to the amount of the corresponding experiment in the orthogonal test table to join the functional monomer 4 - vinyl pyridine, cross-linking agent ethylene glycol dimethyl methacrylate, solvent chloroform, then add the initiator azodiisobutyronitrile toluene solution 3 mL (13 mg/mL). Placed in an ultrasonic cleaner ultrasonic 15min, with 30mL carbon tetrachloride into a three-necked flask, under nitrogen, set the temperature to 60 ℃, power is 200W, reflux confined to a predetermined reaction time. Methanol ultrasonic washing several times into a soxhlet extractor, methanol: acetic acid (9:1) template, reflux extraction with methanol washing finally. 80 ℃ placed in an oven and dried in vacuo, ground spare. Obtain Fe_3O_4 magnetic surface imprinted microspheres. Blank Fe_3O_4 magnetic surface imprinting microsphere preparation without quercetin, the same goes for the rest of the way.

TABLE1 PREPARATION OF FE_3O_4 IMPS

Test No	Time （h）	Quercetin（mg）	4-vp(mg)	Cross-linkers（mg）	Chloroform（mL）
1	1	40	70	34.1	7
2	2	60	42	34.1	6
3	1.5	60	70	44.6	8
4	2.5	40	42	44.6	5
5	1	50	42	55.1	8
6	2	30	70	55.1	5
7	1.5	30	42	23.6	7
8	2.5	50	70	23.6	6
9	1	30	56	44.6	6
10	2	50	84	44.6	7
11	1.5	50	56	34.1	5
12	2.5	30	84	34.1	8
13	1	60	84	23.6	5
14	2	40	56	23.6	8
15	1.5	40	84	55.1	6
16	2.5	60	56	55.1	7

HPLC sample preparation and chromatographic conditions

3.3Sample Preparation

Methanol solution with 2mg / mL ethyl acetate extract of raspberry, take 16 parts, each 30mL were added to 16 parts of Fe_3O_4 magnetic surface imprinted microsphere obtained in 3.2, static adsorption 1h, the supernatant was discarded, Fe_3O_4 magnetic surface imprinted microspheres were used to 70mL water, 70mL30% methanol washing, the eluent was discarded, and finally with 100mL of methanol and methanol washings were collected and concentrated to 2mL, namely HPLC samples.

3.4Chromatographic conditions

DIKMA C18 column was reversed-phase column (250 × 4.6mm, 5 μ m), flow velocity:1mL/min, column temperature:30 ℃ ,Injection Volume:20 μ L, Mobile phase: acetonitrile(A): 0.1% formic acid water(B) (0-70min,A3%-20%;70-90min,A20%-35%;90-105min,A35%-55%;105min,A 55%-100%); Determine wavelength: 266nm.

3.5Adsorption Performance Analysis

Preparation of 0.5mg/100 ml,1.0mg/100ml,1.5mg/100ml,2.0mg/100ml,2.5mg/100ml of quercetin each 100 ml of acetonitrile solution, respectively according to take five 0.4 g, Fe_3O_4 magnetic surface imprinting microspheres and precision according to calm, to join in the above solution, suspension after 1 h of adsorption, static, and magnetic field under the condition of separation, take the supernatant were recorded at 368 nm absorbance values. Static saturated adsorption capacity formula for $Q_e = (C_0 - C_e)$ V/M, including Q_e for adsorption capacity (mg/g) per gram, molecularly imprinted polymer adsorption amount of quercetin; C_0, C_e (mg/mL) respectively before and after adsorption, the concentration of a solution of quercetin V (mL) is the volume of a solution; The quality of the M (g) to join the IMPs. According to Scatchard equation $Q/C_e = (Q_{max} - Q)/K_d$ can determine the maximum equilibrium constant K_d and Q_{max}. Type of Q and Q_{max} equilibrium adsorption quantity and maximum adsorption, respectively, the C_e for the concentration of quercetin in equilibrium solution; K_d to balance the binding sites of equilibrium dissociation constant, to Q/C_e Q mapping, formula into $Q/C_e = (1 / K_d) Q + Q_{max}/K_d$, according to the linear relationship between the slope and intercept of K_d and Q_{max} two parameters can be obtained.

4. Results and Discussion

4.1Result of Fe3O4 magnetic nanoparticles surface oil acidification and electron microscope scanning

From preparation of Fe_3O_4 magnetic nanoparticles and the surface of oleic acid:Synthesis of oleic acid of Fe_3O_4 magnetic nanoparticles all in toluene layer, the aqueous layer is clarified, showing better performance lipophilic Fe_3O_4 magnetic nanoparticles, the surface of the success of oleic acid.

From SEM results, synthesized Fe_3O_4 particles diameter of about 30nm, Fe_3O_4 nanoscale particles of synthetic polymer imprinted higher surface area greater adsorption efficiency.

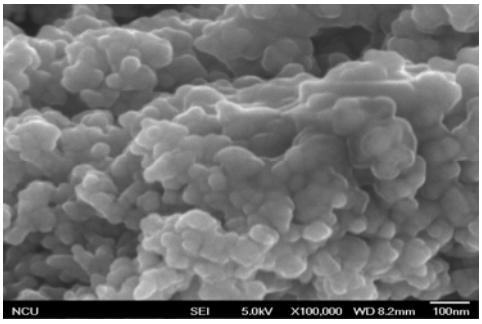

Fig1 Fe_3O_4 SEM-SEI results

4.2Results of Fe3O4magnetic surface imprinted microsphere preparation process analysis

Combined ethyl acetate extract of raspberry fingerprints, Fe_3O_4 surface imprinted microspheres prepared by the specific adsorption of flavonoids peak area and Y1 and non-specific adsorption of the other ingredients of the peak area ratio and Y2 Y1 / Y2 is

screening of the best indicators of preparation; mean Y1/Y2 as shown in Table 3, SPSS17.0 analysis results are shown in Table 2:

TABLE2 ORTHOGONAL RESULT OF FE_3O_4 IMPS

Test No	Y1/Y2	Test No	Y1/Y2	Test No	Y1/Y2	Test No	Y1/Y2
1	3.32	5	4.68	9	4.20	13	4.49
2	3.76	6	5.43	10	3.87	14	4.93
3	4.93	7	5.03	11	4.62	15	3.70
4	4.89	8	6.47	12	4.66	16	5.06

The results of orthogonal experiment showed that the SPSS analysis showed that, Fe_3O_4 magnetic surface imprinted microsphere preparation of the selected factors P values were less than 0.05, indicating that factors significantly. The average value of each factor shows the best conditions for water: microwave time: 2.5h; quercetin quantity: 50mg; 4- vinyl pyridine quantity: 70mg; crossl-inkers amount: 23.6mg; chloroform volume: 5mL. Repeat prepared in accordance with the conditions Fe_3O_4 magnetic surface imprinted microsphere three times to give theY1/Y2 are: 6.75, 6.83, 6.79, were greater than the orthogonal experiment inY1/ Y2.

4.3HPLC fingerprint results

In optimum conditions obtained in preparation 4.2 Fe_3O_4 magnetic surface imprinted microspheres and blank Fe_3O_4 magnetic surface imprinted microsphere, and in accordance with Sample Preparation, resulting samples were analyzed by fingerprinting conditions. The results show (Figure 2): Fe_3O_4 magnetic surface imprinted microspheres can be effectively extracted from ethyl acetate liquid raspberry isolated flavonoids quercetin and other active ingredients. Blank Fe_3O_4 magnetic surface imprinted microsphere only a small amount of non-specific adsorption of components.

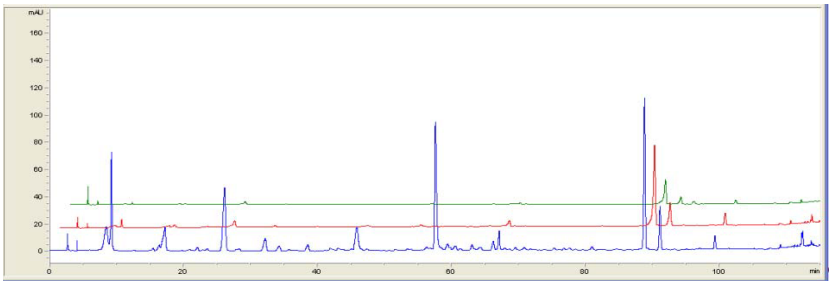

Fig 2 Separation effect Comparison of Quercetin-selective Fe_3O_4 MIPs by Fingerprint

4.4Adsorption analysis

According to the experiment adsorption performance analysis, the adsorption isotherm(fig 3) show that good linear relationship. Scatchard analysis is shown in Figure 4.

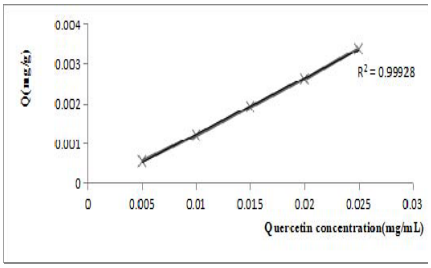

Fig3 Adsorption isotherm

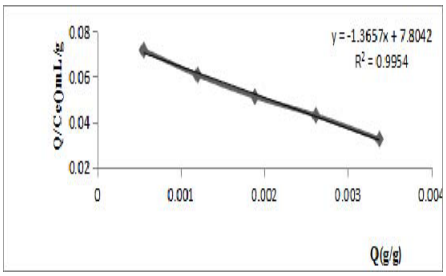

Fig4 Scatchard analysis diagram

Seen from Fig.4,Fe_3O_4 magnetic surface imprinted microspheres Scatchard adsorption curve is essentially a straight line, the correlation coefficient(R) of regression analysis for the curve is 0.9954.It is noted that the quercetin-selective Fe_3O_4 magnetic surface imprinted microspheres has only one distinct section within the plot and inclined to a straight line, which presents that only the homogeneous affinity binding sites are formed in the polymers. Scatchard equation:$Y=-1.3657X+ 7.8042$,$R^2=0.9954$,according to the equation can be calculated equilibrium dissociation constant $K_d=0.7322mg/L$, the maximum adsorption capacity Q_{max} =18.92 μ mol/g. Quercetin silica surface is pre-imprinted polymer prepared by the maximum adsorption capacity of 6.59 times [5].

5. Discussions

Fe_3O_4 magnetic nanoparticles in the preliminary experiment used by co-precipitation method is in uneven size and easy to clog. The study found particle size found in $Fe_3O_4 \cdot 7H_2O$ solution and $FeCl_3 \cdot 6H_2O$ solution diluted 1-fold was in excellent dispersion, surface effect and magnetic effect of Fe_3O_4 magnetic nanoparticles works well. Studies have shown that more dilute solution can ensure a redox reaction more fully.

Microwave radiation make the molecularly imprinted polymer preparation time dozen times shorter than before, this may be caused by microwave radiation reducing the activation energy of the reaction.

The study of Fe_3O_4 magnetic surface imprinting microspheres for inherited Fe_3O_4 superparamagnetism realized in the applied magnetic field and the rapid separation of mother liquor; After 1h of static adsorption, UV detects that the supernatant fluid concentration will no longer change, basic water washes away IMPs nonspecific adsorption of impurities between composition and particle retention fluid. Methanol elution specificity adsorption flavonoids ingredients, adsorption and parsing convenient quickly; Fe_3O_4 magnetic nanoparticles surface effect makes the IMPs adsorption quantity greatly improved; At the same time Fe_3O_4 magnetic surface imprinting microsphere has good physical and chemical properties, which can be recycled. The group with Fe_3O_4 magnetic surface imprinting microspheres for enrichment of ethyl acetate parts of the flavonoids ingredients, good results have been achieved, the team will continue to study and extract molecularly imprinted polymer with enrichment of flavonoids xtracted directly from raspberry.

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