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Synthesis of a Nanostructured Molecularly Imprinted Acrylic acid-Based Network Copolymer as a Solid Sorbent for the Quercetin extraction

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Abstract

A straightforward approach for the extraction of the quercetin was carried out by a nanoporous molecularly imprinted acrylic acid-based network copolymer as asolid sorbent. This technique involves a molecular template (quercetin) which is surrounded by functional monomers and are subsequently co-polymerized in the presence of an excess of the cross linkers. In this process, three-dimensional binding sites are generated that are complementary to the quercetin template in terms of size, shape and position of functional groups. After removal of the imprinting quercetin template, the nanostructured polymer can therefore specifically recognize and re-bind the same or structurally very similar molecules. The synthesized MIP by bulk polymerization was exhibited a good tendency to absorb the quercetin template in a solid phase extraction (SPE) system. The prepared MIP achieved a binding capacity of 169 mg/grfor the quercetin in acetonitrile-water (1:1 v/v) solvent. Imaging by scanning electron microscope (SEM) was carried out to determine the surface morphology of the prepared MIP.

1. Introduction

In the past decades, molecular imprinting techniques have attracted considerable attention to synthesize polymers capable of selectively recognizing to special molecule for extraction and binding to a specific substrate. Quercetin (3,3_,4,5,7-pentahydroxy flavones, Qu) is one of the most common flavonoids and the most bioactive compound in the flavone class which is widespread in the leaves, fruits and flowers of many plants and vegetable kingdom [1]. According to Fig.1, the creation of the molecularly imprinted polymers (MIPs), involves (1) formation of complexes by covalent, non-covalent or semi-covalent interactions between the template or target molecule and functional monomers in a porogenic solvent, (2) formation of highly cross-linked polymer networks in thermal or photo-initiated radical polymerization,(3) removal of the template molecules by elution to create special space for the template molecule within the polymer matrices.

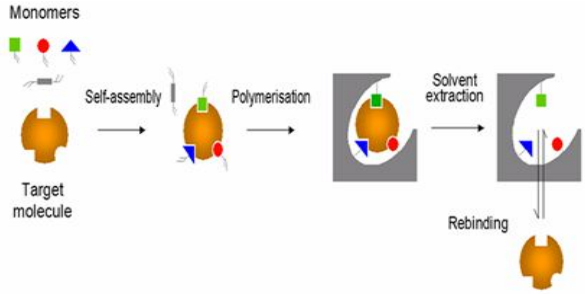

Fig. 1. Principle of molecular imprinting polymer.

Fig.2-4 showsthe 3-dimentional structure of quercetin, acrylic acid (AA) and trimethylolpropane trimethacrylate(TRIM), respectively. There are interactions between AA as a monomer and the quercetin as a template by H-Bonding. This is the main reason to create 3-dimensional space in the matrices of the polymer. The interaction between functional group belong to AA in MIP and the quercetin, cause that these kind of monomers can be used as a functional monomer for effectively performing the molecular imprinting of quercetin [2].

Molecularly imprinted polymers for extraction of the quercetin were synthesized by non-covalent approach in this research [3-6]. Because of The relative ease of preparation, low cost, high se-

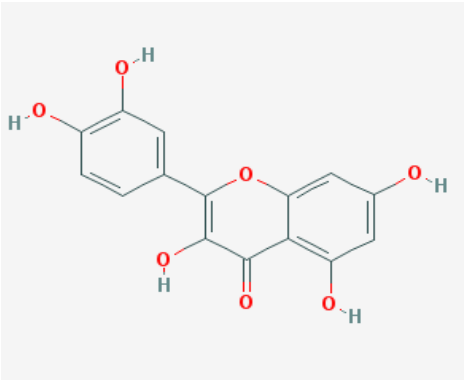

Fig. 2. Quercetin as a template.

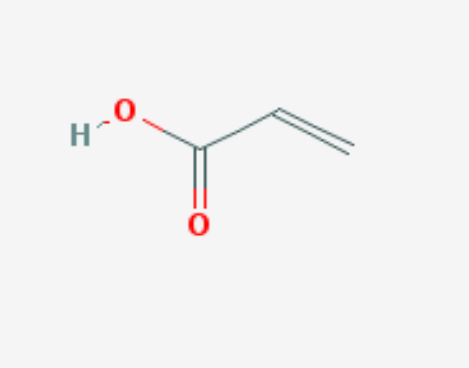

Fig. 3. AA as a monomer.

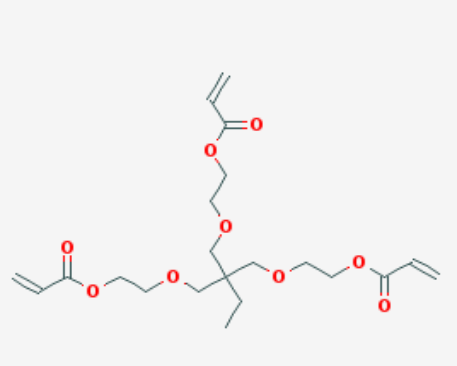

Fig. 4. TRIM as a cross-linker.

lectivity and high sensitivity, MIPs were introduced as a suitable alternative in the field of solid phase extraction [5, 7, and 8].

2. Experimental Procedure

2.1. Methods

The sequences in preparing of molecularly imprinted polymers as a Sorbent material describe as; grinding block polymers after polymerization, sieving, sedimentation in acetone to earn uniform particles and remove fine particles (<5µm);elicit of the template from polymer matrices. After drying of the MIPs, they were tested to measure the binding capacity in a solution containing the quercetin.

2.2. Materials

Quercetin hydrate (Qu), acrylic acid (AA), 2, 2´ azobisisobutyronitrile (AIBN) and trimethylolpropanetrimethacrylate (TRIM) was prepared from Sigma-Aldrich company. Tetrahydrofuran (THF), methanol and acetic acid were prepared in analytical grade.Absorbances were measured at 254 nm by Jenway 6305 UV/Visible spectrophotometers in order to evaluate of the absorbed amount of the quercetin.

2.3. MIP Synthesis

0.03 mmol quercetin hydrate dissolved in 5 ml dry THF as a porogen in a glass vial 18cm length with 2cm diameter. After that, the functional monomer, cross-linker and AIBN as initiator were added in a molecular ratio of 1:8:40 (template :functional monomer :cross-linker).The pre-polymerization solution was prepared in an ice-bath and was dispersed by ultrasound irradiation for three times, once before adding of TRIM to arrange monomer functional group in the presence of the related complementary group in the template to create hydrogen bonding [9] second was done after adding of the cross-linker and third time was carried out after adding of the initiator. The solution was purged with nitrogen for 3 min to remove dissolved oxygen. The polymerization procedure was thermally initiated at 60 °C in a water bath for 16 hrs and was performed at 70 ºC for 3hrs to achieve a solid monolith polymer.

2.4. NIPs Synthesis

Non-imprinted polymer (NIP) was synthesized exactly by the same synthetic route of MIP in the absence of the quercetin template as a control to assess properly the imprinting effect obtained for the target analyte.

2.5. Preparing of the polymers for loading

In order to processing of polymers for application as absorbent matrices, the following steps have been carried out:

1. Removing of the unreacted materials from the surface of solid monolith polymers by solvent.
2. Crush the polymer block to small particles using a porcelain mortar and pestle.
3. The polymer particles were sifted through a 100-mesh sieve.
4. Elimination of fine particles <5 µm was carried out with acetone by sedimentation technique.
5. Drying of the particles in an oven 60 °C for overnight.

2.6. Template removal from MIP

For removing of the template, the prepared polymer has been put in a conical flask containing the eluent(methanol/acetic acid 9:1 V/V) and the solution was continuously stirred with a magnetic stirrer during the extraction. This procedure was allowed till absorbance of the filtered solution in 254nm reach to zero. It means that the entire template has been removed from the polymer. Then MIP was separated from the eluent by centrifuge 11000rpm and washed two times with distillated water. The templet-free MIP was dried at 60 ºC overnight for further use.

3. Results and Discussion

3.1. Characterization of MIP and NIP

Surface morphological information of MIP and NIP was obtained by Atomic Force Microscopy (AFM) Model Easyscan2 Flex (Switzerland), variable pressure SEM model VEGA\\TESCAN-XMU (Canada) and FE-SEM Field Emission Scanning Electron Microscope model S-4160 (Hitachi Japan) instruments. The nonporous cavities in MIP proved by Transmission Electron Microscope (TEM) ZIESS model EM900.

AFM images confirmed that the topography dimensions of MIP (Fig.5)are smaller than NIP (Fig.6).

Imaging by SEM onthe surface of the MIPs was shown in Fig. 7 which indicated the MIPs have the amorphous surfaces.

Probably, the existence of the strong hydrogen bonding between the quercetin and the functional groups in the polymer is the main reason of these properties. Because of this strong hydrogen bonding, noanoporous spaces were created in the MIP which was confirmed by TEM. White Spots in Fig.8, indicated nanoporous cavities in the MIP.

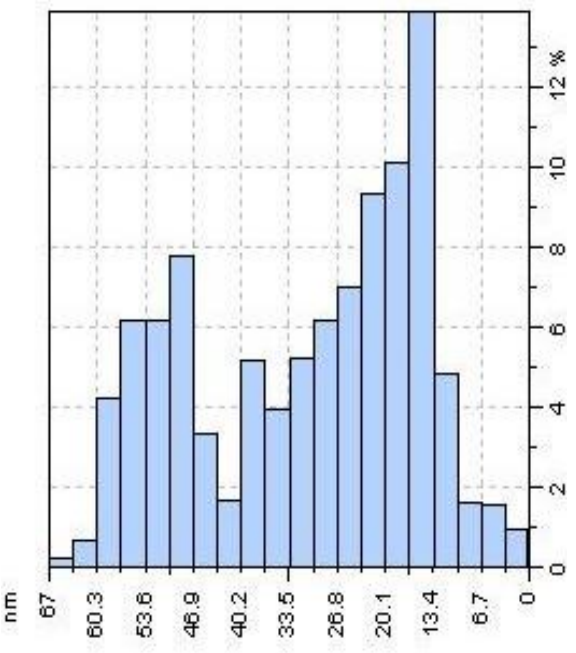

Fig. 5. AFM imaging topography for the MIP

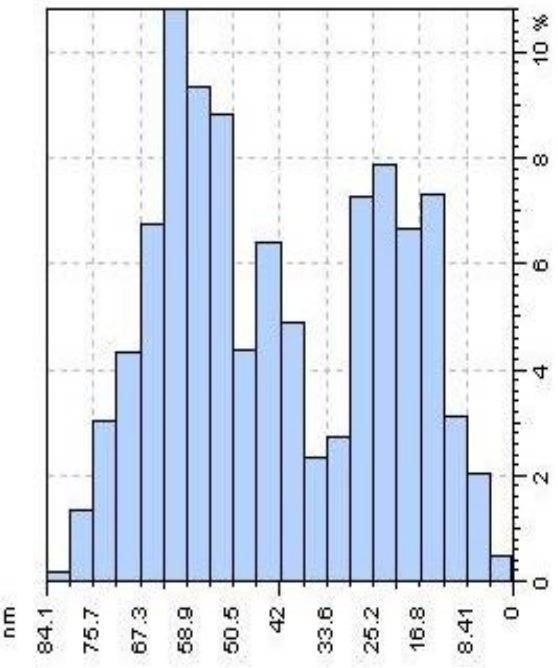

Fig. 6. imaging topography for theNIP

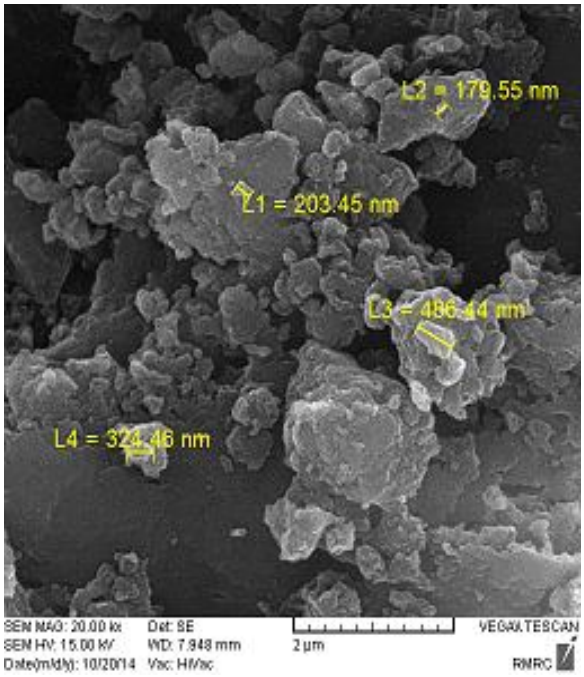

Fig. 7. SEM image for MIPs

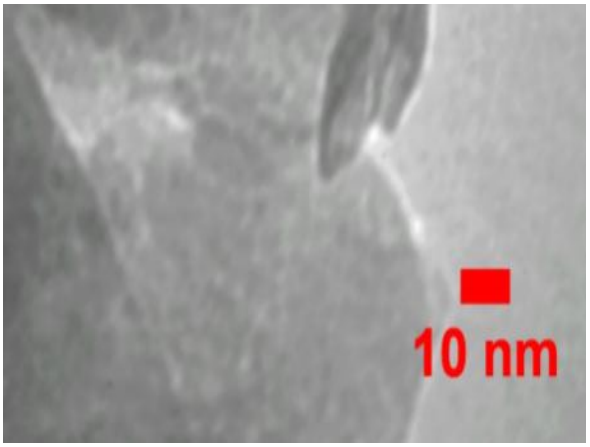

Fig. 8. TEM imaging for MIP

3.2. Binding Studies

The amounts of the extracted compounds were confirmed according to the beer-lambert law and measuring the absorbance at 254nm by a UV-Visible spectrophotometer. The UV spectrum of the quercetin shows that, it absorbs UV light at two different wavelengths, 254 and 380 nm. Since acetonitrile does not absorb light significantly at 254nm, the binding analysis of the prepared MIPs was studied in 254 nm. In order to evaluate the binding capacity of the MIP and the NIP, experiments were conducted at 25°C for three times. Experiments as a function of the quercetin concentration were investigated in static adsorption mode. 10 mg of the leached MIP and NIP was taken in conical flask 50ml, separately. Four different concentrations of the quercetin were prepared in CH_3CN/H_2O (1:1 v/v) solvent. 20ml of the related solution was added to the flask and put on a stirrer for 2hrs at room temperature. In each process, after loading time, the solution was placed in centrifuge tubes and the solid materials was spun down by centrifuge 11000 rpm. 1.5 ml of the supernatant solution was with drawn by the sampler and was transferred to a 2.5ml vial for determination of unloaded concentration of the quercetin by UV-Vis spectrophotometer. The related concentration of the quercetin was calculated according to the equation which has already been earned by the standard absorbance curve of quercetin.

3.3. Binding Capacity (Q)

The binding capacity is defined as the amount of the absorbed template per one gram of the polymer. The binding capacity can be calculated by equation (1):

$$Q= (C_o– C) * V / W \qquad (1)$$

Where, C_o is the initial concentration of the quercetin, C is the concentration of the quercetin after loading time, V is the volume of the feed with initial concentration and W representsthe mass of the polymer.

Table 1. Loading results for the different concentrations of the quercetin in the presence of 10mg of MIP

Experiment no.	1	2	3	4
W(mg)	10	10	10	10
V(ml)	20	20	20	20
Loading Time (hr)	2	2	2	2
C_o (ppm)	111.8	139	391	1050
C(ppm)	85	131	306.5	1024
Q (mg/gr)	53.6	16	169	52
Imprinting Factor (IF)	1.73	2.66	2.41	1.08

Fig.9 shows the binding capacity of MIP for 4 different concentrations ofquercetin.

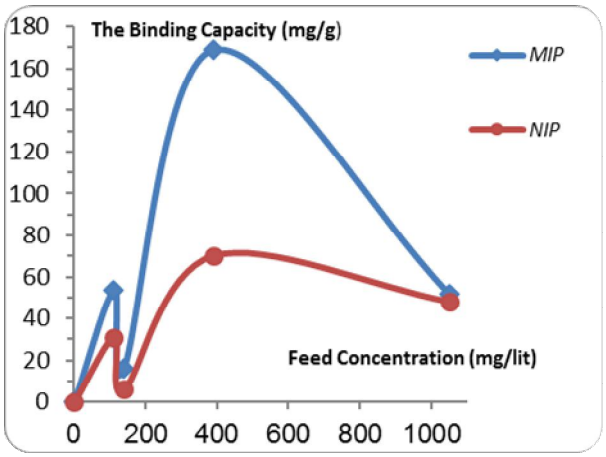

Fig. 9. The binding capacity Vs. C^o

According to the Fig.9, the binding capacity 169 mg/gr was achieved in 391 ppm of the feed concentration. Molinelli et al. [10] reported 0.4mg/gr and Xie et al. [11] reported 12µg/gr of binding capacity for their researches. The functional monomer, cross-linker and some other items in polymerization in this work are different with the above mentioned researches.

3.4. Imprinting Factor (IF)

The imprinting factor can be calculated according to equation 2:

$$IF = Q(MIP) / Q(NIP) \quad (2)$$

The Fig.10 shows that, the optimized IF is 2.41 which pertain to 391ppm of the quercetin concentration.

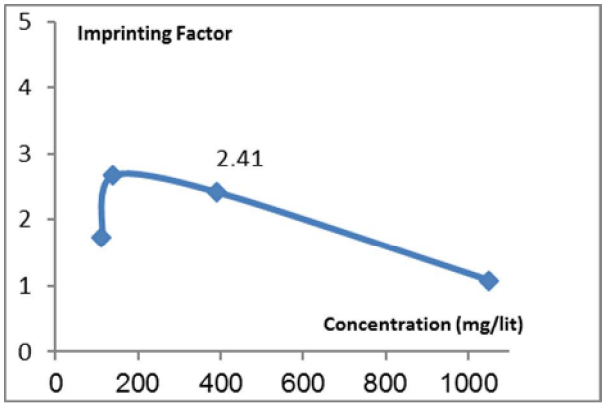

Fig. 10. Imprinting factor Vs.C^o

4. Conclusion

During grinding of the block polymers, it was seen that, the solid monolith polymer of the MIP is harder and more fragile than the NIP, while block of the NIP, are soft with elastomeric specification.

Since all of the process flowchart for synthesis of the MIP and NIP are the same, may be the existence of the strong hydrogen bonding between the quercetin and the functional groups in polymers is the main reason of these properties.Because of this strong hydrogen bonding, noanoporous spaces were created in the quercetin-free MIP which was confirmed by TEM results. White spots in the Fig.10, indicated nanoporous cavities in the MIP.

Since, the MIP is more fragile than NIP, after grinding of the MIP, the related particles will be smaller than NIPs. It was certified from imaging by SEM and AFM.

Scanning electron microscopy of MIP and NIP shows, the related particles have amorphous surfaces.

The binding capacity of 169 mg/gr was achieved in 391 ppm of the feed concentration by UV/Visible method.

The nanoporous polymers prepare the possibility for direct extraction of the certain pharmacophoric components (like quercetin or other molecules) from natural products with MIP technology.

Acknowledgment

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