



The magnetic fructose imprinted polymer for determination of fructose from apple juice

Burcu Okutucu*

Ege University, Faculty of Science, Department of Biochemistry, 35100, Bornova-Izmir, Turkey

Received: 02 February 2018, Revised: 26 April 2018, Accepted: 30 April 2018

*Corresponding author's e-mail address: burcu.okutucu@ege.edu.tr

ABSTRACT

Molecularly imprinted polymers (MIP) are synthetic receptors that have an ability to recognize and select its template molecule from complex matrix. Nowadays many new approaches of MIPs are researched. One of them is the preparing of magnetic MIPs. Magnetic molecularly imprinted polymer (MMIP) nanoparticles can be a candidate for solid phase extraction adsorbent by the porous morphology, narrow size distribution, stable chemical and thermal property. The aim of this study is to prepare MMIP nanoparticles for solid phase extraction of fructose from apple juice. The PEG treated Fe_3O_4 magnetic nanoparticles surface was coated with monosaccharide (fructose) imprinted polymer. The monomer acrylamide, cross-linking agent ethylene glycol dimethacrylate (EGDMA), initiator azobisisobutyronitrile (AIBN), the porogen dimethylsulfoxide were used for fructose imprinting. The structural characterization of MMIP was performed by FT-IR, and the imprinting characteristics of polymer were also studied by adsorption experiment and Scatchard analysis. The 65% of fructose was recognized with fructose MMIP from apple juice using only an external permanent magnet without filtration/centrifugation.

Keywords: Nanoparticles, magnetic molecularly imprinted polymers, fructose, apple juice.

1. INTRODUCTION

Molecularly imprinted polymers (MIP) are synthetic receptors that have a highly specific recognition ability for target molecule. MIPs are produced by target molecule, monomer(s), crosslinker together with in the porogen by thermal or UV polymerization. After the target molecule is removed, the cavities of polymer can rebind target molecule on size, shape and functionality¹. Nowadays the widespread use of molecularly imprinted polymer in separation, chemical catalysis, chemical sensing has increased, and also to combine them with

Manyatik fruktoz baskılanmış polimer ile elma suyundan fruktoz tayini

ÖZ

Moleküler baskılanmış polimerler (MIP) karışık ortamlardan kendi kalıp molekülünü tanıma ve ayırt etme yeteneğine sahip sentetik reseptörlerdir. Günümüzde MIP'in bir çok yeni uygulaması araştırılmaktadır. Bunlardan birisi manyatik MIP'lerin hazırlanmasıdır. Manyatik moleküler baskılanmış polimerler (MMIP) poroz yapıları, belirli boyut aralıkları, stabil kimyasal ve termal özelliklerinden dolayı iyi bir katı faz ekstraksiyon adsorbantı için iyi bir aday olabilir. Bu çalışmanın amacı, elma suyundan fruktozun katı faz ekstraksiyonuyla elde edilmesi için MMIP nanopartiküllerini hazırlamaktır. Bunun için çalışmada PEG ile kaplanan Fe_3O_4 manyatik nanopartiküllerinin yüzeyi monosakkarit (fruktoz) baskılanmış polimer ile kaplandı. Monomer akrilamid, çapraz bağlayıcı etilen glikol dimetakrilat, insiyatör azobisisobutirilonitril ve porojen olarak dimetilsülfoksit fruktoz bakılama için kullanıldı. MMIP'in yapısal karakterizasyonu FT-IR tarafından gerçekleştirildi ve polimerlerin baskı özellikleri de adsorpsiyon deneyi ve Scatchard analizi ile incelendi. Fruktozun % 65'i, filtrasyon/santrifüjleme olmaksızın sadece bir harici sabit mıknatıs kullanılarak elma suyundan fruktoz MMIP ile tanındı.

Anahtar kelimeler: Nanopartikül, manyatik moleküler baskılanmış polimerler, fruktoz, elma suyu.

different materials is an interesting and attractive area. Magnetic nanoparticles were one of these materials. There are many different possible approaches that magnetic nanoparticle MIP can be used. The usage of magnetic nanoparticles as solid phase extraction (SPE) adsorbents are very widespread. The advantages of using magnetic adsorbents in SPE are removing of any desired molecules from complex sample matrices by applying an external magnetic field. This process is very rapid and convenient. When some magnetic nanoparticles are used with MIPs as a core, the magnetic MIPs (MMIPs) are occurred. These materials can have not only magnetically

characteristic, but also selectivity for the guest molecule. And also they have a controllable rebinding process because of the characteristics of the molecularly imprinted polymers. The usage of MMIPs provides a separation faster and easier because of removing by an external magnetic field without centrifugation or filtration. Recently, magnetic polymers have many areas that can be used especially in medicine, biotechnology, and environment.²⁻¹⁰

Magnetic iron oxide or magnetite, Fe_3O_4 , is used commonly as the magnetical component in molecularly imprinted polymer technique because of its low toxicity, ease of preparation (even if in large-scale production), ease of operation (the magnetic particles can be removed easily from the bulk solution by applying an external magnetic field), ease of surface modification with different functional groups, excellent dispersibility in aqueous solution and low cost.¹¹

The preparation of magnetic MIPs is done by self-polymerization of monomers with target molecules at the surface of the magnetic nanoparticle core. The most important point is the choice of monomers. Because the aim is the preparation of a thin MIP layer on the surface of magnetic nanoparticles. Mostly acrylamide and methacrylic acids are used. The acrylamide provides multiple hydrogen-bonding sites as well as hydrophilicity, thus imprinted polymer template-monomer complexation can be formed by hydrogen bonds, which is the most found in imprinted polymers.¹²⁻¹⁵ Molecular recognition of the template molecule in imprinted polymer is based on intramolecular interaction between the template and functional groups in the polymer which is found by the polymerization of monomer and crosslinker. So, crosslinker have to be used to contribute to template complexation and polymer morphology.

In this study, Fe_3O_4 magnetic imprinted polymers were prepared for fructose. It was acrylamide used as a monomer, ethylene glycol dimethacrylate as a crosslinker and DMSO as a porogen. Characterization studies and Scatchard analysis were also done.

2. MATERIALS AND METHOTDS

2.1. Materials

Acrylamide, fructose, ethylene glycol dimethacrylate (EGDMA), methanol (MeOH), polyethylene glycol (PEG), chloroform (CHL), dimethylsulfoxide (DMSO), ammonium hydroxide (25%) were obtained from Sigma (USA). Azobisisobutyronitrile (AIBN) was purchased from Wako Pure Chem. Ind. (Japan). Sulfuric acid was taken from Fluka. All other chemicals and reagents were of the highest available purity and used as purchased.

2.2. Preparation of magnetic fructose imprinted polymeric nanoparticles

The preparation of magnetic fructose imprinted polymeric nanoparticles was divided into two steps. The first step is the synthesis of PEG- Fe_3O_4 magnetic nanoparticles by co-precipitation method. 1 mol l^{-1} $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$ and 0.75 mol l^{-1} $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$ and 80 ml distilled water was placed into three-necked flask (clear yellow solution). The mixture was stirred at 500 rpm and 80°C for two hours under N_2 pressure. After two hours 0.4 M ammonium hydroxide (28%, in percent weight) was added dropwise to the solution and (the color of the solution was turned to black) continuing to stir at 500 rpm for 30 minutes.¹⁶ The magnetic nanoparticles were separated from the solution by magnetic separator. The resulting magnetic nanoparticles were washed with 3 times distilled water and 2 times methanol. After washing, magnetic nanoparticles were obtained and dried under vacuum at 45°C for further usage. For surface modification, 500 mg of magnetic nanoparticles were put in 2g PEG / 20 ml distilled water and stirred for two hours. To obtain homogeneous PEG- Fe_3O_4 nanoparticles, the solution was put into the ultrasonic bath for 45 minutes and centrifuged at 5000 rpm for 20 minutes.

The second step is imprinting process. The prepolymerization solution acrylamide (30 mg) and fructose (20 mg) was dissolved in DMSO (5 ml) and waited for 30 minutes for prepolymerization. The PEG- Fe_3O_4 nanoparticles were placed into three-necked flask and firstly prepolymerization solution (monomer-template) was added to the flask and then EGDMA (0.9 ml) and 10 mg AIBN were added and stirred. To obtain fructose imprinted PEG- Fe_3O_4 nanoparticles, the mixture was stirred under N_2 pressure at 800 rpm for 30 minutes, and then continued to stir 500 rpm for 4 hours at 60°C and for 20 hours at 25°C in the same speed. By the help of magnetic separator, fructose imprinted PEG- Fe_3O_4 nanoparticles were taken from the solution and was ready for subsequent usage. The magnetization of magnetic fructose imprinted polymer obtained is shown in [Figure 1](#). The control magnetic nanoparticles were prepared without fructose (NMMIP). The MMIP and NMMIP were used directly for extraction without grinding or sorted by size.



Figure 1. Magnetization of magnetic fructose imprinted polymer.

2.2.1. Elution of template (fructose)

The resultant fructose imprinted PEG-Fe₃O₄ nanoparticles were washed repeatedly with methanol:acetic acid (4:1) solution and 2 times methanol at 25°C to remove the fructose. The complete removal of fructose from the fructose imprinted PEG-Fe₃O₄ nanoparticles was confirmed by o- cresol: H₂SO₄ assay.¹⁷ The standard range of fructose was between 1 and 300 μmol ml⁻¹.

2.3. Binding experiments

2.3.1. Static adsorption experiments

Adsorption experiments were performed to evaluate the recognition properties of MMIPs at different conditions (pH, substrate concentration, polymer content, etc.). The adsorption capacity of magnetic imprinted nanoparticles towards fructose was assayed with rebinding experiments. Briefly, 10 mg of magnetic polymers (template removed) was mixed with different concentration of fructose (1-50 μmol ml⁻¹) in 1 ml of methanol in an eppendorf tube and shaken for 2 h at orbital rotator and at room temperature. After that time, the magnetic polymers were centrifuged at 5000 rpm for 5 minutes and the concentration of the remaining or unbound fructose in the solution was determined by o- cresol: H₂SO₄ assay. The NIP was used as a control to determine the non-specific binding. The amount of fructose (Q; μg g⁻¹) bound to the polymers was calculated by subtracting the amount of free substrate from the initial concentration. The amount of fructose that is imprinted is defined by Eq. (1).

$$Q = (C_0 - C_t) \times V / W \quad (1)$$

Where W (g) is the weight of the magnetic nanoparticles, V (l) is the volume of solution (methanol), C₀ (μmol l⁻¹) (initial concentration of the fructose) and C_t (μmol l⁻¹) (concentration of fructose at the supernatant) respectively. All of the measurements were carried out in triplicate, and the average values were calculated.

2.3.2. Scatchard analysis

To evaluate binding sites of fructose imprinted polymer (scatchard plot analysis) was used in a batch mode. Briefly, 5 μmol ml⁻¹ of fructose concentration were studied with different amount of imprinted and non-imprinted magnetic nanoparticles (10-100 mg) in 1 ml methanol and was shaken for 2 hours. After that time, the tubes were centrifuged and the supernatant was analyzed by o- cresol: H₂SO₄ assay.

The binding parameter of fructose imprinted magnetic nanoparticles was estimated by Scatchard analysis by using Eq. (2).

$$Q_e / C_e = (Q_{max} - Q_e) / K_d \quad (2)$$

Where K_d (μmol ml⁻¹) is the equilibrium dissociation constant, C_e (μmol ml⁻¹) is the equilibrium concentration of fructose, Q_{max} (μmol g⁻¹) and Q_e (μmol g⁻¹) are the apparent maximum adsorbed amount and the equilibrium adsorbed amount of fructose, respectively.

2.4. The application of fructose MMIP to real sample

The preparation of apple juice for fructose analysis with MMIP is studied. The apple is an important fruit for children and adults. As known, it is a rich source of sugars and biologically active compounds. The sugars presented in apple (juice) are fructose 5-15% (w/w), glucose 1-14% (w/w), sucrose 1-5% (w/w).¹⁸ The traditional gravimetric, chemical and instrumental methods for carbohydrate analysis are destructive, expensive, sophisticated and time-consuming. Magnetic MMIPs are a good alternative for fructose in a complex medium. The apple sample was prepared for fructose magnetic MMIP. The apple was ground and then the juice was extracted using a fruit juice extractor. The liquid was filtered through Whatman No.54 paper. The fructose content was determined by o- cresol: H₂SO₄ assay (6.56 g ml⁻¹).

To evaluate the specific recognition ability of fructose MMIP; 1 ml of apple juice was immersed with 20 mg of MMIP for 10 minutes. After the treatment of magnetic nanoparticles with the external field, the upper solution was analyzed (Unbound). The MMIP was washed with methanol and, the bound content was analyzed. The all fructose samples were tested with o- cresol: H₂SO₄ assay.

3. Results and Discussion

3.1. The preparation of fructose imprinted magnetic nanoparticles

The most common method for preparing Fe₃O₄ particles were chemical co-precipitation method. This method has many advantages as mentioned in the following: For example, this method is the simplest and most effective way of obtaining homogeneous nanoparticles, and also magnetic nanoparticles can be coated or grafted with starch, dextran, PEG, PVA, and so on. If the magnetic nanoparticles are not coated with surface coating material, their surface becomes

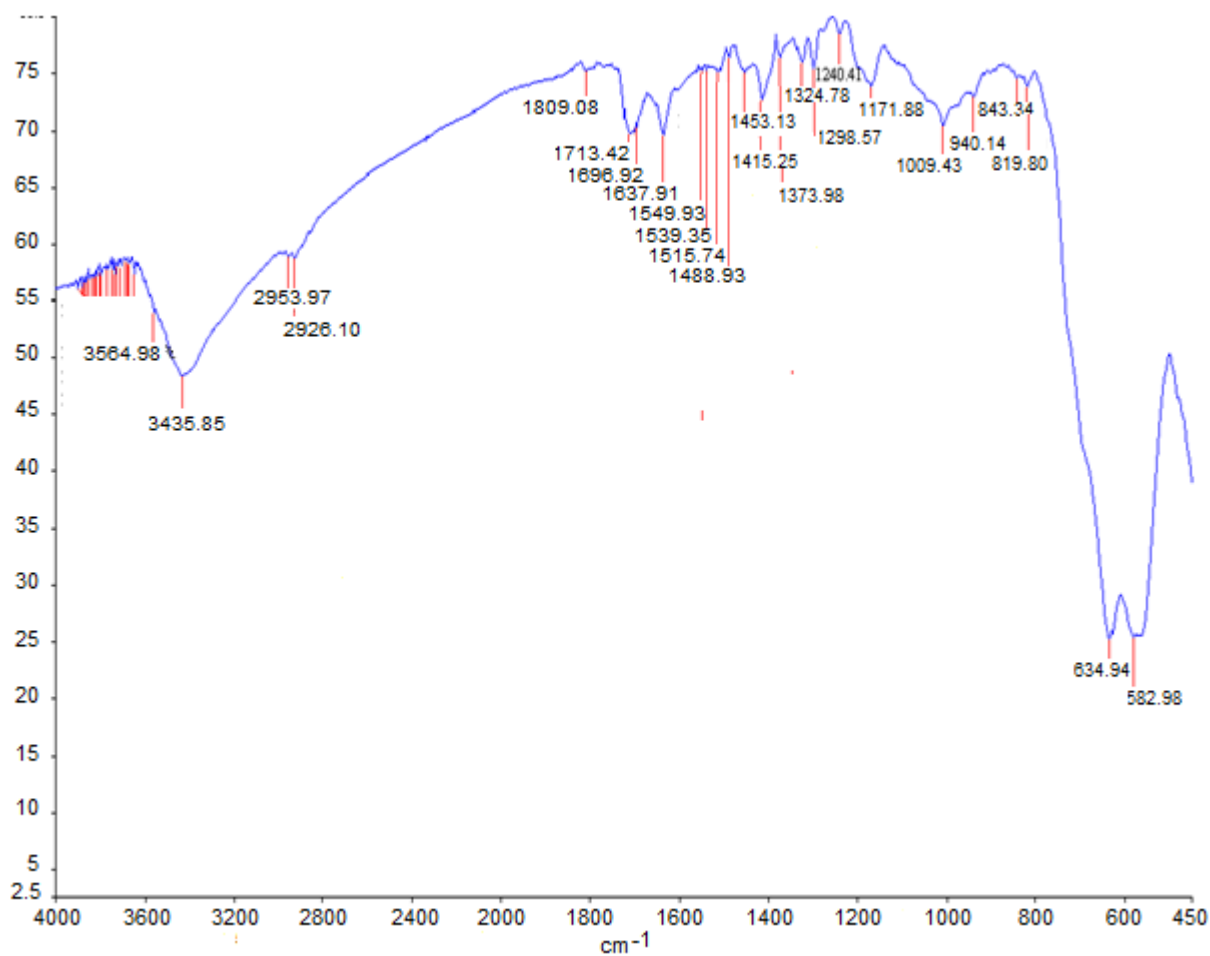


Figure 2. FT-IR of MMIP.

hydrophobic. As a result of this hydrophobicity, the particles agglomerate and form large clusters (increased particle size leads to lose of the magnetic properties, etc). The advantages of coating are to prevent aggregation, and this also renders the nanoparticles water soluble or oil-soluble, and provides functionalization for the conjugation of biomolecules. In this study, PEG was used as the surface coating material to introduce a polymeric chain on the surface of the magnetic nanoparticles. Molecular recognition of the template molecule in imprinted polymers is based on the intermolecular interaction between the template molecule and functional groups in the polymer chain. Many of the monomers were tested at different properties (acidic, neutral, basic), but the best result was obtained by acrylamide. The monomer selection is a very important part of preparing MMIPs. Because monomer directly affects the imprinted layer thickness, which has a significant influence on the imprinting factor. Also the apparent thickness was the sign of maximum rebinding capacity and imprinted sites. As known, a porogenic

solvent can affect the morphology and recognition capability of the MMIPs. Usually, polymerization is done in the low-polar solvent which has advantages. The magnetic imprinted polymers prepared using acrylamide with DMSO as porogenic solvent had better molecular recognition. When DMSO was used, good homogeneity and density of the resultant beads could be prepared reproducibly. Also fructose (template molecule) was solved in DMSO very well. The other important factor for the taken best imprinting factor was molar ratios between the functional monomer and cross-linker. The most effective ratio was the ratio of monomer to cross-linker of 1:2 (i.e. acrylamide: EGDMA ratio), which was found in the experiment, and also this ratio gives high adsorption capacity and selectivity for fructose MMIPs. It can be explained that if there is not enough crosslinker, effective imprinting sites cannot be formed. On the other hand, too much cross-linker will lead to the template molecules being embedded too deep and will reduce effective imprinting sites.

On the other hand, FT-IR analysis was performed to obtain better informed about the preparation of magnetic fructose MMIPs (Figure 2). Compared with Fe_3O_4 nanoparticles, the absorption band of Fe-O at 582 cm^{-1} in MMIPs proved that Fe_3O_4 was embedded in these materials. The characteristic peak of acrylamide was observed at 3435 cm^{-1} , indicating the N-H is stretching of acrylamide.

3.2. The adsorption graphs and Scatchard analysis

The rebinding of fructose on MMIPs was studied. As shown in Figure 3, the binding amount of fructose to the magnetic polymers increased until it reached a saturation level. The results are shown that the specific cavities to fructose is existed and to lead higher adsorption capacity.

In order to obtain the binding affinity of polymers and their theoretical number of binding sites for the template, the Scatchard equation was used. It is an effortless and straightforward way to recognize multiple classes of binding sites and provides a graphical presentation of binding data. The K_d and Q_{\max} values were calculated with the help of Eq. (2). The K_{d1} $4.65\text{ }\mu\text{mol}^{-1}$, $Q_{\max1}$ $29\text{ }\mu\text{mol ml}^{-1}$, and K_{d2} $37.6\text{ }\mu\text{mol}^{-1}$, Q_{\max} $2.53\text{ }\mu\text{mol ml}^{-1}$ values were found, respectively.

The results of these values show that; the binding site configuration in the MMIP is heterogeneous, and indicates that the binding sites can be classified into two distinct groups with different specific binding properties. As seen from Figure 4, there are two distinct linear sections suggesting two binding sites. One is of high selectivity and the other is of low affinity. The origin of molecular recognition in MIPs can be generally attributed to both shape selectivity and pre-organization of functional groups, however external magnetic field also effected the contributions of these effects. Because of this reason; there are two different binding sites.

3.3. The selectivity study of fructose MMIP at apple juice

The selectivity of magnetic fructose imprinted polymer nanoparticles is tested with apple juice. The amount of initial fructose in apple juice was determined by o- cresol: H_2SO_4 assay. After applying the apple juice to MMIP and NMMIP, the solution was also tested with o- cresol: H_2SO_4 assay to determine the adsorbed amount of fructose. The ratio of C_p (the amount of fructose adsorbed on magnetic fructose MIP, as $\mu\text{mol g}^{-1}$) to C_s (the equilibrium concentration of fructose in solution, as $\mu\text{mol ml}^{-1}$) was the fructose that is determined by MMIP. As seen in Table 1, magnetic fructose MIP can recognize fructose by a 65% ratio from the complex matrix (i.e. apple juice). NMMIP can recognize only 10% of fructose because of surface functional groups of magnetic MIP.

Table 1. The selectivity of fructose with magnetic fructose imprinted polymer from apple juice

	Fructose Content (g ml^{-1})
Conventional method (o- cresol: H_2SO_4 assay)	6.56
Mangetic Fructose imprinted polymer	4.25
Non-imprinted polymer	0.6

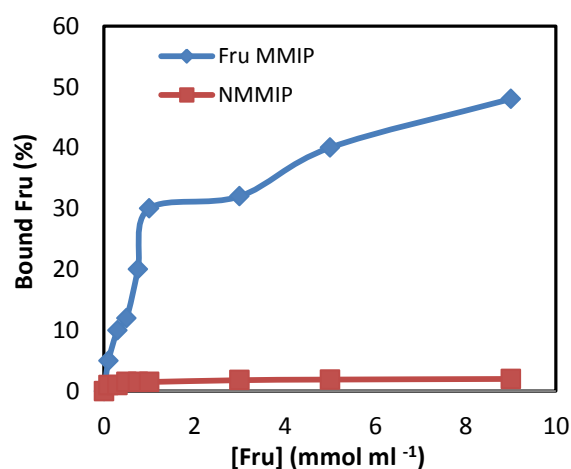


Figure 3. Adsorption graphs of magnetic fructose imprinted (Fru MMIP) and non-imprinted (NMMIP) polymer.

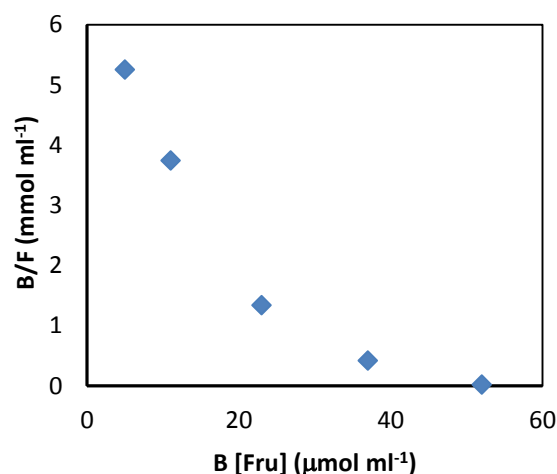


Figure 4. A Scatchard graph of magnetic fructose imprinted polymer.

The fructose of apple juice was also determined by conventional method (o- cresol: H₂SO₄ assay). However, before the assay applied, the apple juice was centrifuged, and then was filtered twice to get a clear solution with the aim to analyze. The results taken from MMIP were without centrifugation. By this result, fructose MMIP can be effectively used to analyze fructose from apple juice.

4. CONCLUSIONS

Molecularly imprinted polymers with magnetic properties are used as a cheaper and robust alternative to separate from complex media. The characterization of fructose imprinted magnetic polymers was done by FT-IR. The adsorption graphs and the real sample application were investigated. The results indicated that the prepared MMIPs showed a high adsorption capacity and good selectivity to fructose. The main advantage of this fructose MMIPs is the possibility to be stored and used at room temperature without any loss in separation capacity. In this study, the fructose can be analyzed from apple juice without any extra analytical methods such as filtration or centrifugation. It was shown that magnetic fructose MIP can be an alternative adsorbent for solid phase extraction.

ACKNOWLEDGEMENTS

This study was financially supported by Ege University Scientific Research Foundation (Project No: 2011 FEN 042).

Conflict of interest


Authors declare that there is no a conflict of interest with any person, institute, and company, etc.

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ORCID

 0000-0002-0907-4175 (B. Okutucu)