Synthesis and Evaluating of Nanoporous Molecularly Imprinted Polymers for Extraction of Quercetin as a Bioactive Component of Medicinal Plants

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ABSTRACT: *In this work, the template, monomer and cross-linker with the ratio of 1:8:40 were used to synthesize Molecularly Imprinted Polymers (MIPs) for extraction of the bioactive chemical compounds from some traditional herbs as a sorbent material. Quercetin, Methacrylic Acid (MAA), Trimethylolpropanetrimethacrylate (TRIM) and Tetrahydrofuran (THF) were used as a template, functional monomer, cross-linker and porogen, respectively. Polymer particles have been evaluated by Atomic Force Microscopy (AFM), Field Emission Scan Electron Microscopy (FESEM), Transmission Electron Microscopy (TEM) and Brunauer–Emmett–Teller (BET). The produced nanoporous MIPs, with a good specific surface area 167.899 m^2/g comparatively to Non-Imprinted Polymers (NIPs), exhibited a good affinity to quercetin with binding capacity of 392.08 mg/g in acetonitrile-water (1:1v/v). The results showed that the MIPs can be used as a sorbent. Thus, direct extraction of certain pharmacophoric components from herbal plants is considerable by MIPs technology.*

KEYWORDS: *Molecularly imprinted polymers; Quercetin; Medicinal plants; Nanoporous; Extraction; Bioactive.*

INTRODUCTION

Recently, molecularly imprinting techniques have attracted attentions to synthesize polymers with selectively recognizing to special molecule for extraction. Successful applications in the separation and purification of the expensive natural medicines for MIPs were caused; the researchers have focused their attention to the direct separation of high value active ingredients from traditional medicine [1]. Separation of the bioactive components from herbs by molecularly imprinting technique is a highly efficient and cost-effective method [2, 3]. Meanwhile, the relative ease of preparation, low cost, high selectivity and sensitivity, along with superior

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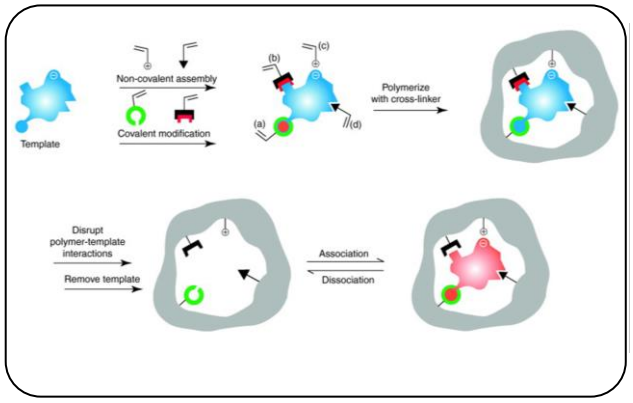

Fig.1: Schematic representation of the molecular imprinting process.

chemical and mechanical properties, MIPs are introduced as a good alternative to natural receptors for a variety of applications in solid phase extraction, separation, synthetic binding assays, biomimetic catalysts, drug delivery and sensing applications [4-9]. Among all of the applications, we are particularly interested in extraction of quercetin from medicinal plants. Quercetin is one of the most common flavonoids and bioactive chemical compound in the flavone class and there is widespread in the vegetable kingdom, leaves, fruits and flowers of many plants such red Onions, Ginkgo, Calendula persica [10]. Nowadays, quercetin is a topic of increasing interest based on its various bio activities such as prevention of radiation induced oropharyngeal mucositis in patients with cancers, antiviral and antitumor properties [11-14]. Because of the existence of quercetin in nature at low concentrations and its structure is similar to other flavones, its separation from other compounds is very difficult. Typically, the creation of MIPs involves three general steps: **(1)** formation of complexes by covalent, non-covalent or semi-covalent interactions between the template molecule and functional monomers in a porogenic solvent, **(2)** formation of highly cross-linked polymer networks *via* thermal or photo-initiated radical polymerization, **(3)** removal of the template molecules from polymers by eluent to create special space (nanoporous) within the polymer matrices. According to Fig. 1, the functional groups of the templates are surrounded by the functional groups of the monomers.

During the polymerization procedure, the monomers are bonded to each other around the template. The cross-linkers complete the polymerization reactions to produce 3-D polymer matrices. Hence, un-leached MIPs are produced. If the related templates are extracted by the eluent, the leached MIPs will be prepared. The leached MIPs will be contained 3-dimensional binding cavities that are known as nanoporous space. These MIPs are able to absorb the same templates or very similar molecules based on shape, structure and stereospecificity. So, they can be used as sorbent materials in solid phase extraction. Due to its closest resemblance to natural bio-molecular interactions such as hydrogen bonding, non-covalent imprinting has been widely used for most of the templates molecules including amino acids, peptides, sugar derivatives, drugs, pesticides, proteins, and other constituents [15-18]. In this research work, two kinds of the MIPs with the ratio 1: 8: 40 by non-covalent approach were synthesized. A good binding capacity achieved for quercetin from the molecularly imprinted MAA-based network polymers (MIPs-MAA). The structure of the MIPs was comparatively characterized by Fourier Transform Infrared (FT-IR) spectroscopy, SEM and BET analysis using non-imprinted molecular polymers as control. Nanoporousity was seen by TEM imaging and confirmed by BET evaluations. The results for MIPs-MAA with a good specific surface area 167.899 m^2/g demonstrated that, extraction of the bioactive components from herbal plants can be suggested by MIPs technology.

EXPERIMENTAL SECTION

Methods

Synthesis and evaluation of the molecularly imprinted block-polymers was carried out in 3 steps: (1) the preparation of fine particles of the MIPs and NIPs [19]. (2) Elution of the template from particles of the polymers by MeOH/AcOH (9:1, v/v) to achieve blank MIPs. (3) Loading on the blank MIPs by quercetin in acetonitrile-water (1:1v/v) in batch mode to measure the binding capacity of the MIPs. Two kinds of the MIPs were synthesized from two different monomers Acrylic Acid (AA) and MAA in ratio 1:8:40. All of the other materials and methods were similar together.

Materials

Quercetin hydrate, 2,2´Azobisisobutyronitrile(AIBN), AA, MAA and TRIM were purchased from Sigma-Aldrich company. All of the solvents such as THF, methanol and acetic acid were used HPLC grade. The absorbance

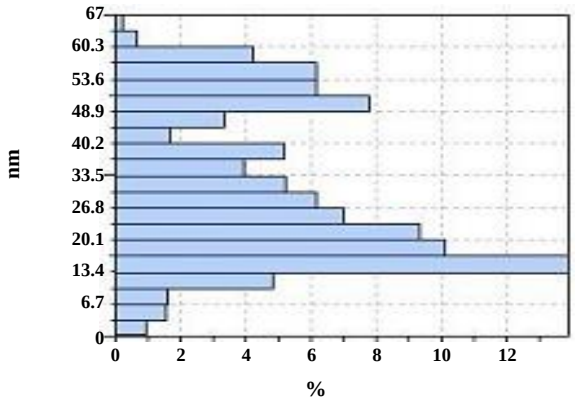

Fig. 2: Roughness and Particles sizes distribution of the MIPs-AA.

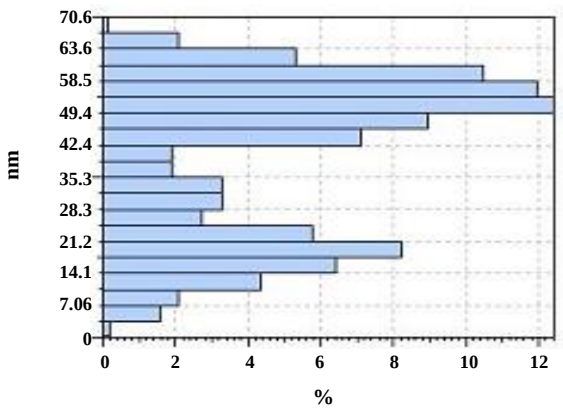

Fig. 3: Roughness and Particles sizes distribution of the NIPs-AA.

was measured in 254 nm by Jenway 6305 UV/Visible spectrophotometer to evaluate of the binding capacity.

MIPs Synthesis

0.03 mmol quercetin hydrate was dissolved in 5 mL dry THF in a glass vial with 18 cm length and 2cm diameter. The functional monomer and cross-linker were added in a molecular ratio of 1:8:40 (template: functional monomer: cross-linker). 2 mg initiator AIBN was added and the solution was put in ice-bath. The pre-polymerization solution was sonicated by ultrasonic waves, three times [20]. The solution was purged with nitrogen for 3 minute to remove dissolved oxygen. The polymerization was thermally initiated at 60 °C in a water bath for 16h and performed at 70 ºC for 3h to achieve a solid monolith polymer. The ratio of template molecule: functional monomer can be varied and it is effective in binding capacity value. Shan *et al.* reported the ratio 1:4 in preparation of molecularly imprinted polymers for improved Quercetin Recognition [21].

NIPs Synthesis

NIPs were synthesized as a control polymer, by the same synthetic routes in the absence of the template molecules to assess properly the imprinting effect obtained for the target.

Preparation of the polymers for loading

The following steps were carried out for processing of the polymers (NIPs & MIPs) as absorbents:

1. The surface of the block polymers was cleaned by porogen solvent to remove un-reacted materials.

2. The block polymer was crushed by porcelain mortar and pestle.

3. The crushed particles were sieved by using 100 mesh sieves.

4. The sieved particles has been washed with acetone to eliminate fine particles of grain sizes <5 μm.

5. The particles of MIPs were eluted by to gain leached MIPs.

6. The particles were dried in the oven at 60˚C overnight.

Elution of the template from MIPs

The prepared polymers were eluted by methanol/acetic acid (9:1 V/V) with a magnetic stirrer. This procedure was allowed up to the absorbance of the filtered solution in 254nm reach to zero. It means that the entire template has been removed from the polymers. The MIPs were centrifuged and washed two times with distillated water. Then they were dried at 60 ºC overnight to give the leached MIPs.

RESULTS AND DISCUSSION

Morphological Studies

Surface morphological information of Molecularly ImPrinted AA-based (MIPs-AA) network polymers and Non-ImPrinted AA-based (NIPs-AA) network polymers was obtained by using AFM model Easyscan2 Flex (Switzerland),Variable Pressure SEM model VEGA\\TESCAN-XMU (Canada) and FE-SEM model S-4160 (Hitachi Japan) instruments.

Fig. 2 and Fig. 3 show the distribution of particles sizes of MIPs and NIPs. The template is captured inside polymer matrices and occupied space, so the leached

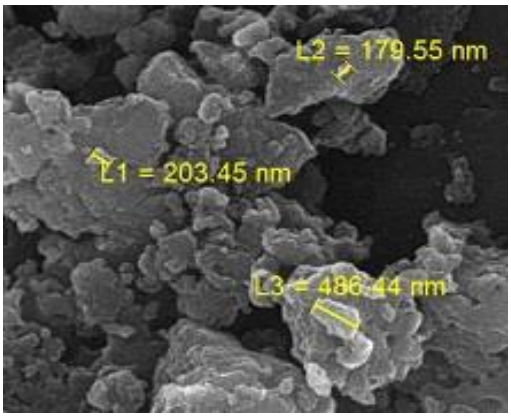

Fig. 4: SEM surface morphology of the MIPs-AA.

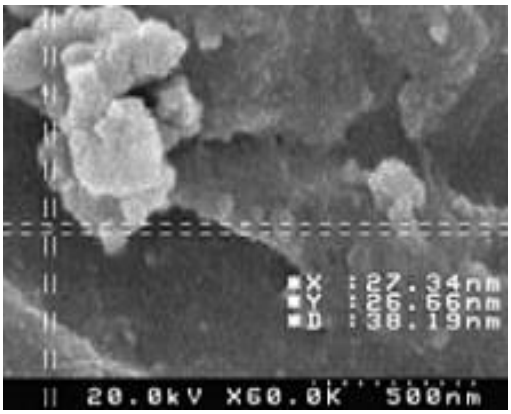

Fig. 5: FESEM surface morphology of the MIPs-MAA.

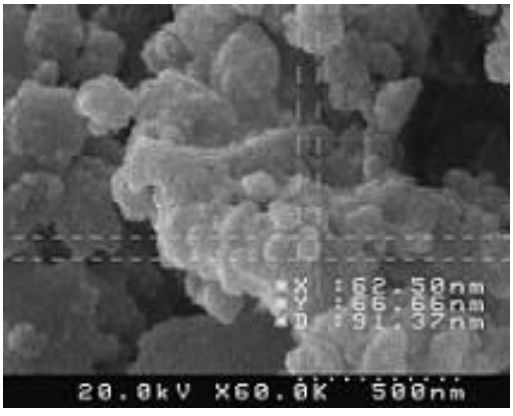

Fig. 6: FESEM surface morphology of the NIPs-MAA.

MIPs will be cancellous and more fragile than NIPs. Thus, after crushing by pestle, it is expected that the roughness and Particles sizes on MIPs to be smaller than NIPs.

Figs. 4-6 show the surface imaging of the MIPs and NIPs which were synthesized by AA and MAA monomers. Surfaces of the MIPs-AA are more rigid while Surfaces of the MIPs-MAA seems softer and more cancellous. It can be one of the reasons that MIPs-AA has low binding capacity than MIPs-MAA. There is no significant difference between surfaces of MIPs-MAA and NIPs-MAA.

H-bonding study

Nano pores in molecularly imprinting polymers are created resulting in the strong hydrogen bonding between the functional groups in monomers and the template. Quercetin molecule contains five phenolic hydroxyl groups that are both the ordinary hydrogen bond donor and the ordinary hydrogen bond acceptor and at the same time contains one carbonyl group that is the acceptor of the ordinary hydrogen bond (Fig. 7).

There is a great deal of hydroxyl groups in polymers that act both as donor and acceptor of the ordinary hydrogen bond in the matrices of the MIPs, whereas carbonyl groups of the carboxyl, act as the ordinary hydrogen bond acceptor (Fig. 8).

Hydrogen bonding between carbonyl groups of the polymer and hydroxyl groups of quercetin in un-leached MIPs, decrease the strength of the carbonyl groups, so its stretching vibration will be appeared less than the related frequency in the leached MIPs. The C=O stretching vibration of the carboxyl groups is 1710-1780 (s) cm^{-1}. FT-IR spectra (400–4000 cm^{-1}) were prepared on a Perkin–Elmer FTIR 1720x spectrometer with 2 cm^{-1} resolution using 20 scans to recognize the related hydrogen bonding. Fig. 9 shows the FT-IR spectra for NIPs-MAA, leached and un-leached MIPs-MAA.

In this figure, the C=O stretching vibration for un-leached MIPs, NIPs and leached MIPs, are closely 1717, 1720 and 1724 cm^{-1}, respectively. It means that, there is not H-bonding between leached MIPs and quercetin. In other words, there is not quercetin in binding sites; therefor the carbonyls groups are free in the matrices of the polymers.

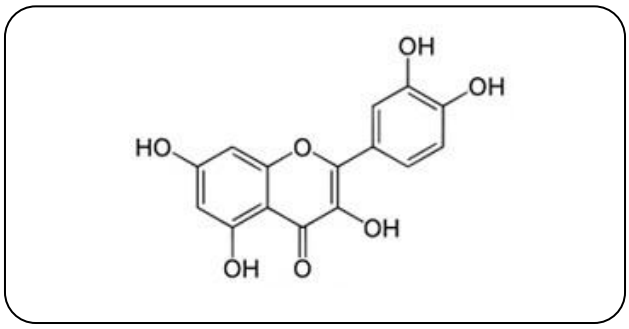

Fig. 7: Quercetin structure.

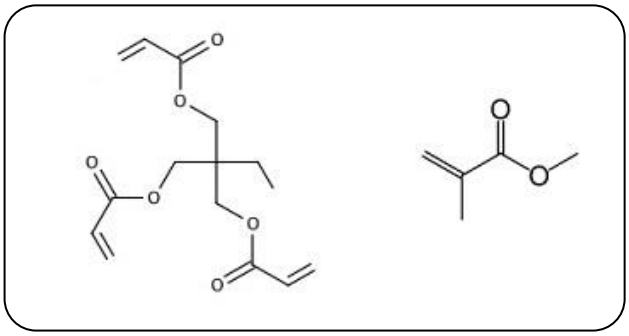

Fig. 8: TRIM and MAA structures.

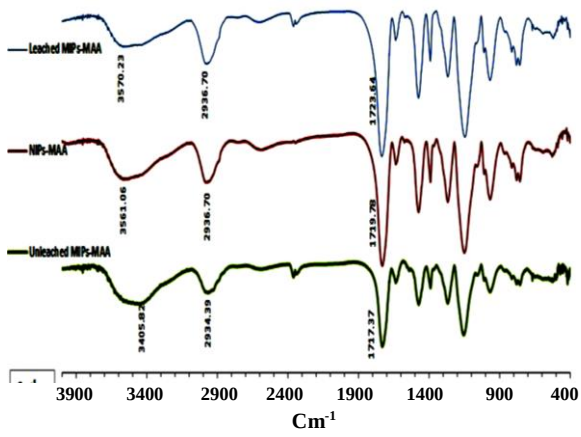

Fig. 9: IR spectra of un-leached MIPs, NIPs and leached MIPs.

Binding Studies

UV spectrum of quercetin shows that, quercetin absorbs UV/Visible light at two different wavelengths 254 and 380 nm, where the band at 254 nm is attributed to the benzoyl chromophore and the band at 380 nm is assigned to the cinnamoyl. Since acetonitrile does not absorb significantly light at 254 nm, the binding analysis of the MIPs was studied by UV/Visible spectrophotometer analysis in this wavelength. Three times experiments were conducted at 25°C in order to evaluate the binding capacity of the MIPs and NIPs. Experiments on adsorption as a function of quercetin concentration for the MIPs and NIPs were investigated in static adsorption mode. In this process 10 mg of MIPs and NIPs was taken in conical flask 50 mL, separately. Four different concentrations of quercetin were prepared in acetonitrile-water (1:1 v/v) solvent. 20 mL of the related solution was added to the flask and put on stirrer for 2 h at room temperature. In all experiments, after loading time, solution was placed in centrifuge tubes and the solid material was spin down by centrifuge 11000 rpm. The supernatant solution (1.5 mL) was withdrawn by sampler and transferred to a small vial for the determination of the concentration of quercetin. We tried our best to separate polymer particles from the solution to measure concentration of quercetin by UV/spectrometer, accurately. Concentration of quercetin was calculated via standard absorbance curve of quercetin.

Calculation of the binding Capacity (Q):

Binding capacity was defined as mg of substrate bound per gram of polymer. The binding capacity can be calculated by the equation (1):

$$Q= (C_o– C) * V / W \quad (1)$$

where, C_o is the initial concentration, *C* is the free concentration of quercetin in supernatant, *V* is the volume of the feed with initial concentration of quercetin and *W* represents the mass of polymer in grams in loading procedure. Different values of the binding capacities of polymers for quercetin have been reported: 0.325 mmol/g [15], 120 µmol/g [20], 12 µg/g [22] and 0.4 mg/g [23]. In all of their researches work, MIPs and NIPs were synthesized in bulk polymerization technique. *Qiu et al.* reported 0.011mmol/g of the binding capacity for the MIPs synthesized *via* precipitation polymerization [24]. According to Fig. 10, the binding capacity 392.08 mg/g was achieved for MIPs-MAA in batch mode and 400 ppm of the feed concentration. A 20 mL solution containing different concentration of quercetin was treated with the polymers (10 mg) at 298 K by magnetic stirrer for 2h.

The results are comparative to the similar researches. It should be notified that, the functional monomer, cross-linker and some other items in the polymerization

Table 1: Comparing parameters of synthesized MIPs by two kinds of monomer.

Kind of polymer	$C_\circ$ (ppm)	C (ppm)	Q (mg/g)	Imprinting Factor(IF)
MIPs- MAA	108.00	88.85	38.30	2.06
MIPs- MAA	250.00	175.00	150.00	1.36
MIPs- MAA	400.00	203.60	392.08	1.32
MIPs- MAA	723.00	555.74	334.52	1.28
MIPs- AA	111.00	85.00	53.60	-
MIPs- AA	139.00	106.50	65.00	-
MIPs- AA	391.00	306.50	169.00	-
MIPs- AA	1050.00	1024.00	52.00	-
NIPs-MAA	108.00	98.72	18.56	-
NIPs-MAA	250.00	195.00	110.00	-
NIPs-MAA	400.00	251.98	296.04	-
NIPs-MAA	723.00	593.34	259.32	-

Table 2: Standard Curve equation for measuring the template.

Template	Kind of Polymer	Usage	Equation	Regression Coefficient (R^2)
Quercetin Quercetin	MIPs-MAA	binding study	y = 0.0649x + 0.0033	0.985
	MIPs-AA	binding study	y = 0.091x + 0.0259	0.997

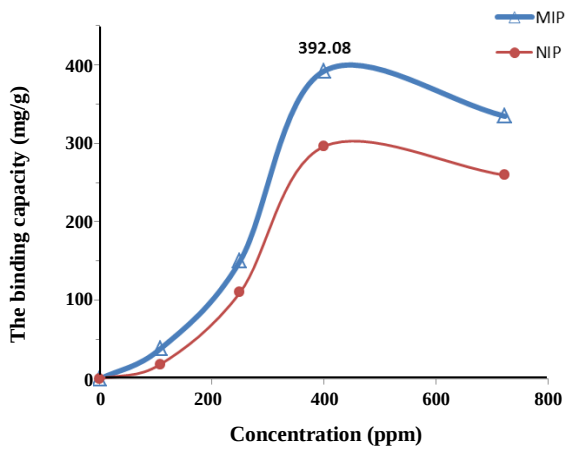

Fig. 10: The binding capacity Vs. Concentration for MIPs-MAA.

procedures were different from the above mentioned researches. Table 1 show that, in this ratio of polymerization, the binding capacity of the MIPs-MAA, is more than MIPs-AA. May be, the methyl groups in MAA have desorption with other groups and resulting in steric effects is caused MIPs-MAA to be more cancellous than MIPs-AA with more porosities.

Hence, MIPs-MAA will have more ability to absorb quercetin relative to MIPs-AA.

The concentration of quercetin was calculated *via* standard absorbance curve of quercetin (Table 2).

Imprinting Factor (IF):

The imprinting factor can be defined by the Equation (2):

$$IF = Q\ (MIPs) / Q\ (NIPs) \quad (2)$$

where, *Q (MIPs)* and *Q (NIPs)* are the binding capacity of MIPs and NIPs, respectively.

Fig. 11 shows, variation of the imprinting factor according to concentration of quercetin for MIPs-MAA.

The related imprinting factor for concentration of 400 ppm is 1.32.

Porosity Analyses and Recognition

The inner structure and the porosity of the MIPs have been detected by TEM ZIESS model EM900. White spots in Fig. 12 indicate nanoporous sites in MIPs.

There is not any white spot in Fig. 13. It means that, nanoporous was just produced in MIPs. These sites are special space to absorb the related templates base on their structure.

The related vacancies, average pore size, specific surface area and pore volume, were characterized by nitrogen gas adsorption measurements using BET analysis (PHS1020-China). Fig. 14 and Fig. 15 show BET reports of the MIPs-MAA and NIPs-MAA. The related measuring indicated the specific surface area of MIPs-MAA was 167.899m^2/g while the specific surface area of NIPs-MAA was 93.545m^2/g. Besides, the micropore specific surface area for MIPs was 183.548m^2/g while the micropore specific surface area of the NIPs was 116.275m^2/g. It means that the nanopores in MIPs have clearly been created more than NIPs. High porosity in MIPs causes the binding capacity to be increased.

Nanoporous materials are a subset of porous materials, typically having large porosities and pore diameters between 1- 100 nm. The average pore diameter of MIPs and NIPs which were measured by BET method, were 0.257 nm and 0.464 nm respectively (Table 3).

So these kinds of the polymers are classified as a micropore of nanomaterials.

CONCLUSIONS

The best binding capacity and specific surface area of this research was 392.08 mg/g and 167.899m^2/g, respectively which it appertained to MIPs-MAA. Surface morphology imaging of the MIPs and NIPs by AFM and SEM indicated that, distribution of the particles sizes of MIPs and NIPs are different together. MIPs by other monomers and cross-linker (ethylene glycol dimethacrylate) with other ratio have been reported

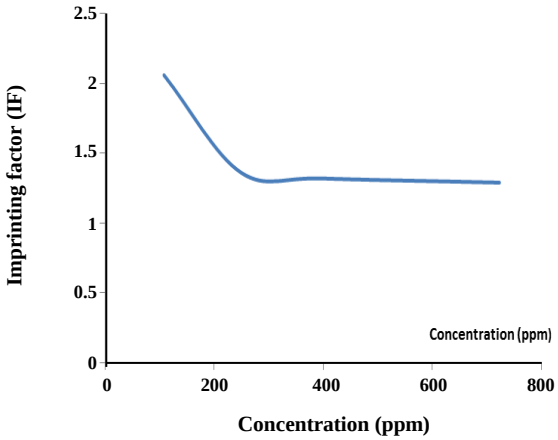

Fig. 11: Imprinting factor Vs. Concentration.

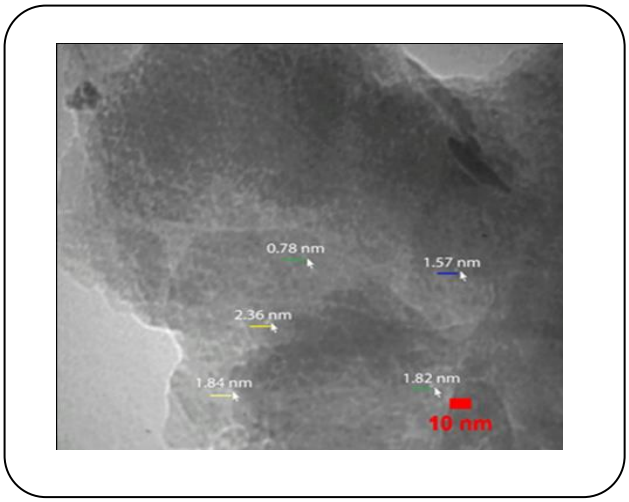

Fig. 12: White Spots diameter in TEM imaging for MIPs.

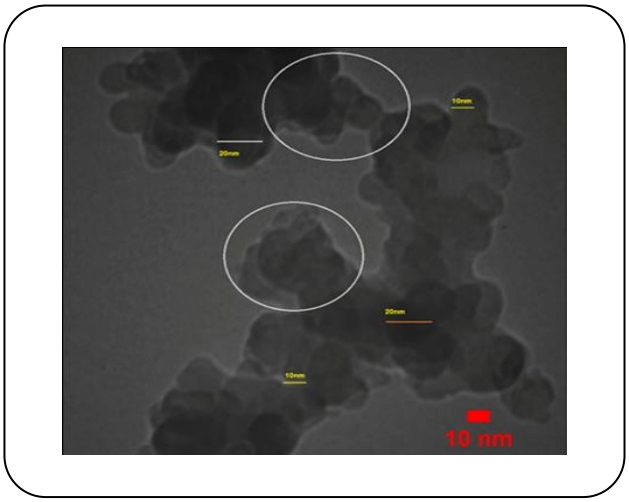

Fig. 13: TEM imaging for NIPs without white spots.

Table 3: BET analyses of the MIPs-MAA and NIPs-MAA.

Kind of polymer	The BET Specific surface area (m^2/g)	The Langmuir Specific surface area (m^2/g)	Micropore specific surface area (m^2/g)	Average pore diameter of MP model(nm)
MIPs- MAA	167.899	649.878	183.548	0.257
NIPs-MAA	93.545	289.741	116.275	0.464

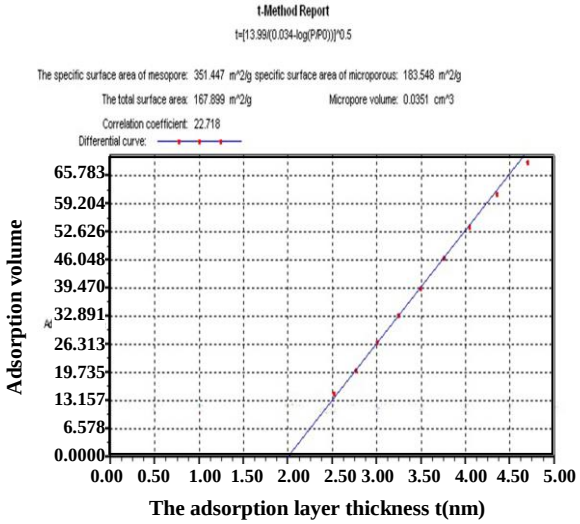

Fig. 14: BET analysis of the MIPs-MAA (T-Plot).

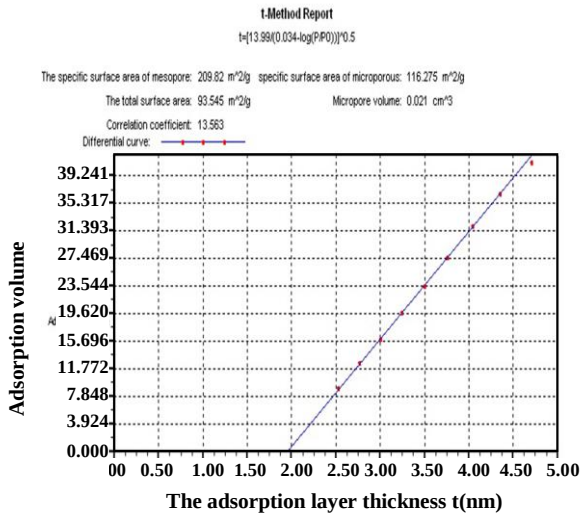

Fig. 15: BET analysis of the NIPs-MAA (T-Plot).

but producing of molecularly imprinted MAA-Based network polymers with TRIM has been carried out for the first time. BET and TEM analysis confirmed that, nanoporous MIPs were produced. These vacancies will be able to adsorb special molecules. Hence, MIPs technology is a good candidate for extraction of the bioactive components from herbal plants. Optimizing to earn the best binding capacity for MIPs in extraction of quercetin is important, so we are interested in to continue our researches. Consideration of selectivity for above mentioned MIPs and synthesis of the MIPs for extraction of the other bioactive components from medicinal plants are our future schedule.

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