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RESEARCH ARTICLE

Molecularly Imprinted Solid-phase Extraction for the Selective Determination of Ciprofloxacin Designer Drugs in Pharmaceutical Preparations

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Abstract

We have developed a new method with high sensitivity, low cost and high stability. Using a functional monomer, this method is based on a molecular polymer (MIP).Which is 2 (Trifluoromethyl) acrylic acid (TFMA), 3 (Trimethoxysilyl) propylmethacrylate (TMSA),suitable cross linker and ciprofloxacin (CPF) template for the production of monolithic solid phase micro extraction (SPME) fiber. Used_ UV. Vis_ as (detector).All these analytical methods used to extract, preconcentration and selective Ciprofloxacin determination (CPF). Competence, The stability and duration of the manufactured fiber plays a fundamental and indispensable role in SPME. The aim of this study is to examine the factors influencing the polymerization and extraction procedures, as well as to explain the selectivity of the manufactured fiber in the CPF solution. Also related and unrelated compound selectivity .Under optimum conditions. For five patients repeated experiments for three measurements the relative standard deviations) RSD percent are range of at (10-50)ppm of CPFH)Percentage (2.57-3.25). The relative recoveries obtained for CPF are within the range of (95.40 - 99.28) in spiked human urine samples.

Keywords: *Ciprofloxacin / Molecularly imprinted polymer solid-phase extraction / UV. Vis.*

Introduction

Ciprofloxacin (CPF) is widely used to treat a number of bacterial infections [1].CPFH residues are well known to persist in edible tissues that are harmful to human health .Different methods for detecting CPF such as high-performance liquid chromatography have been well established [2, 4], Spectrophotometry [5, 6], electrophoresis of the capillary zone [7] or micellar liquid chromatography [8].

These analytical techniques, however, require expensive instrumentation, complex pretreatment of samples, and preparation, which cannot meet actual large-scale testing [9, 10]. It is therefore highly desirable to use a fast, inexpensive but sensitive method to detect CPF residues. Molecularly impressed polymer (MIP) is a rapidly developing technique for synthesizing molecular recognition materials (MIP) with tailor-made selective recognition sites over the last few decades [11, 13]. Due to its unique characteristics, MIP is widely used in different analytical methods such as colorimetric sensors [14], drug detection [15], extraction [16], chromatographic separation [17], and catalysis [18] etc. A simple and useful method is the electrochemical deposition of MIP film on an electrode. This method has many advantages such as controlling film thickness, easily growing and adhering to any size and shape transducer, simplicity, and high sensitivity[19].To our knowledge, however, electrochemical sensors based on MIPs have rarely been reported for CPF determination so far.

It was determination some drugs such as ibuprofen [20], war far in sodium [21] and Methamphetamine [22]. In this work, we focused on developing a highly selective molecularly impressed sensor to overcome the inconvenience of conventional CPF detection methods. In this work using molecularly impressed polymers (MIPs) for solid phase extraction (MISPE) enables quick and selective extraction compared to traditional methods.

Experimental

Chemicals and Apparatus

2 (Trifluoromethyl) acrylic acid (TFMA),3 (Trimethoxysilyl) propylmethacrylate (TMSA), Trimethylolpropane triacrylate (TMPT) and benzoyl peroxide(BP) were purchased from Sigma-Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com), ethanol, acetonitrile, acetic acid, were purchased from Merck (Darmstadt, Germany, www.merck.com).) Ciprofloxacin (CPF) was provided from the medico legal institution (Baghdad, Iraq). Nitrogen gas (99.99) from Arab gulf factory Baghdad.

Instrumentation

Monitoring of the analytes was performed using a UV-Vis (Shimadzu UV spectrophotometer 1800 pc (Japan))and Scanning electron microscopy (SEM) (JSM.6390A) (TOKYO JAPAN) and FTIR Shimadzu (FTIR) - 8000 (Japan), heating /string (Germany) and centrifuge(Germany).

Chromatography is a technique used to separate a mixture of ingredients into its original components and to use a solid phase micro extraction (SPME) tube to achieve a three-step separation process. Start with the sample injection in the GC, the second step is to separate the sample into separate components, and the third step involves detecting the components of the sample using a detector.

Pure Ciprofloxacin absorption at 258 nm, known to remove all Ciprofloxacin after washing, can be used during the polymerization process to ensure that all Ciprofloxacin has been removed and then measured using Agilent 7890A GC The polymer solution was stirred with an ultrasonic bath (SONERX) (W.GERMAN).

MIP Preparation Procedure

Template 4.0 mmol (CPF) has been dissolved in 10 mL acetic acid and 5 mL porogen (chloroform) and 40 mmol (TFMA) has been added. Added 160 mml cross linker (TMPT) and 0.5 mg initiator (benzoyl peroxide) after ultrasonically stirring the resulting mixture for 20 min.

Dissolved in 10 mL porogen (chloroform) and 15.0 mmol functional monomer (TMSA) was added to the solution. The resulting mixture was added to the solution after ultrasonically stirring for 20 min, 120 mmol crosslinkers (TFMA) and 0.5 mg initiator (benzoyl peroxide). The two solutions were bubbled for 10 min with nitrogen and used as a pre polymer solution, Rubber sealed the tube.

Then at 55c overnight, the tub was left in the water bath. The polymerization process was completed and the formation of CPF- MIPs was completed. The non-molecular polymer NIP was the same method of synthesis MIP but without the CPF. Several times the MIP and NIP tubes were washed with an excess of a mixture containing acetic acid/acetonitrile (40:10, v / v) in the Soxhlet extraction device for (48h) until the template and unreacted compounds were removed.

Remove as much as possible and dry in vacuum for 1 hour. The prepared MIP and NIP left for daring in the oven. The sampling device used as extraction needles before extraction. Using a plastic syringe, the plastic syringe (Colum) was packed with prepared (MIP). The solution (urine or standard solution) was poured from the top of the column; the solution was moved at 100 rpm by electrical vacuum.

Sampling

Stock solutions were prepared at pH 7 concen tration (10, 30, 50, 70 ppm) of (CPF) and passed through the Colum at a flow rate of 100 rpm. The Colum extraction was washed twice using 5 mL of distilled water to remove the interference from the matrix and then removed (MIP).

The Sampling Device

The device consists of the 5mL plastic syringe and was packed with MIP (0.1, 0.3, 0.5) gm that was previously ground and then sieved with bore size (1.25 µM).

Procedure of Extraction Method

Using a MIP Ciprofloxacin solid phase extraction (SPE) column, ciprofloxacin was extracted from the drugs. Previously, this column was prepared with MIP (0.1, 0.3 g), its reservoir size, three ml. The SPE vacuum was charged with the supernatant taken with flow rate (100 rpm) from the centrifuged urine sample.

1mL of distilled water and 1mL of acetonitrile / distilled water (70:30, v / v) were added to the column and the eluent collected in the small beaker after elution. It was then dried for 20 minutes and 10 mL of acetic acid/acetonitrile (40:10, v / v) was added and the eluates were also collected in

the same beaker and the residue was dried again at 55 ° C in the water bath. The residue was then mixed with 100µl of this solution and heated the sample at 55 ° C for 10 minutes with stirring.

The solution was cooled to room temperature and subsequently evaporated under nitrogen stream the solvent to dryness, 1mL of ethanol was added to residues and ready for injection into GC MC.

Preparation of Pharmaceutical Samples

To obtained the powder of tablets pharmaceutical samples using pestle and mortar to grinding the tablets then taken a suitable weight for preparation in 50 mL of solutions.

Used appropriate amount of acetic acid/ ethanol for dissolved pharmaceutical samples and complete to 50 mL in the volumetric flask by solvent as well as using the magnetic stirrer for more than 10 minutes.

After that filtered the solution by using 0.07µm cellulose filter paper for preparing and obtained the concentrations of 70ppm Ciprofloxacin

Results and Discussion

Synthesis of MIPs for Ciprofloxacin (CPF)

Two CPF MIPs were synthesized by the method of self-assembling (non covalent) bulk polymerization. Functional monomer was an important role in the study of the template interactions. For the synthesis of MIPs and NIPs, two monomers were used, 2 (Trifluoromethyl) acrylic acid (TFMA), 3-(Trimethoxysilyl) propyl methacrylate (TMSA).

FTIR Analysis

FTIR is an important method of chemical cha racterization for the detection of functional gr oups in a compound. Table 1 shows the FTIR spectra of various MIPs and NIPs.

Table 1: The most identified peaks of FT-IR spectra for CPF-imprinted polymer and NIP using TFMA as a functional monomer

	Functional Group	CPF –MIP (TFMA) before template removal	CPF –MIP (TFMA) after template removal
1	N-H str. (cm^{-1})	3269	----
2	O-H str.(cm-1)	3400	----
2	C-H aliphatic.(cm^{-1})	2850,2933	2860,2931
3	C=O str.ester.(cm^{-1})	1631,1776	1650
4	C=C str. (cm^{-1})	1587	1579
5	C-H aromatic.(cm^{-1})	3074,3051	-----
6	C-H bending (cm^{-1})	1471	1463

The Fourier transmission infrared spectrometry spectra of leached and un leached Ciprofloxacin (CPF) imprinted polymers MIP and NIP were recorded in the range of 400-4000 cm^{-1} by the KBr pellet method (Table 1).From the table1,the FTIR spectrum of the CPF shows the following bands: (3269, 3400, 3074and 3051) cm^{-1} for N-H stretching , O-H stretching, C-H. aromatic stretching. The FTIR spectrum of the MIP (TFMA) after template removal shows the absence of N-H stretching , O-H stretching, C-H. aromatic stretching which excise in template (CPF) spectrum which indicate the extracted of drug from template. When using the 3-(Trimethoxysilyl)propyl methacrylate (TMSA).as monomer for synthesis of another MIPs for Ciprofloxacin (CPF), the FTIR spectra of MIPs before and after template removal and NIP are shown in Table 2.

Table 2: The most identified peaks of FT-IR spectra for CPF-imprinted polymer and NIP using (TMSA) as a functional monomer

	Functional Group	CPF –MIP (TMSA) before template removal	CPF –MIP (TMSA) after template removal
1	N-H str. (cm^{-1})	3321	----
2	O-H str.(cm-1)	3380	----
2	C-H aliphatic.(cm^{-1})	2790,2900	2810,2920
3	C=O str.ester.(cm^{-1})	1657,1710	1680
4	C=C str. (cm^{-1})	1560	1550
5	C-H aromatic.(cm^{-1})	3025,3070	-----
6	C-H bending (cm^{-1})	1510	1490

From the Table 2, the FTIR spectrum of the CPF shows the following bands (3321, 3380, 3025, 3070,) cm-1 for N-H is stretching, O-H is stretching, C-H .Aromatic stretching. The FTIR spectrum of the MIP (TMSA) after template removal shows the absence of N-H

stretching, O-H stretching, C-H .Aromatic stretching which excise in template (CPF) spectrum which indicate the extracted of drug from template. Several experiments were conducted using various ratios (D: M: C) to achieve the optimum ratio for MIPs (CPF) preparation. Among these molar ratio experiments (D: M: C) of (1:10:40), (1:10.50) for CPF -MIPs have produced polymers suitable characteristics list in the Table3.

Table 3: The variation ratios of [D: M: C] and progeny used in the preparation of MIPs and NIPs for (CPF)

		Drug CPF	Monomer (TFMA)	Cross linker TMPT	Initiator	Solvent
MIP	%	1	8	40	0.5	10 mL acetic acid
	M mole	0.08	0.64	3.20	0.5	/ acetonitrile
MIP	%	1	6	50	0.5	10 mL acetic acid
	M mole	1.10	6.60	55.00	0.5	/ acetonitrile
MIP	%	1	10	40	0.5	10 mL acetic acid
	M mole	2.00	20.00	80.00	0.5	/ acetonitrile
NIP	%	----	10	40	0.5	10 mL acetic acid
	M mole		20	80	0.5	/ acetonitrile
		Drug CPF Mmole	Monomer (TMSA)	Cross linker TMPT	Initiator	Solvent
MIP	%	1	10	40	0.5	10 mL acetic acid
	M mole	1.50	15.00	46.00	0.5	/ acetonitrile
MIP	%	1	15	50	0.5	10 mL acetic acid
	M mole	2.00	30.00	100.00	0.5	/ acetonitrile
MIP	%	1	10	50	0.5	10 mL acetic acid
	M mole	2.00	20.00	100.00	0.5	/ acetonitrile
NIP	%	----	10	50	0.5	10 mL acetic acid
			20.00	100.00	0.5	/ acetonitrile

Adsorption Isotherm

Isotherm adsorption is useful to understand the adsorption mechanism of the polymer surface adsorption template. Data from isotherm adsorption equilibrium were analyzed to show the typeof Langmuir isotherm or Freundlich models [23 - 24]. This was determined by plotting the ability to bind (Q) against the drug's free concentration, Q is calculated as follows:

$Q = [(C_i - C_f)\ V_s * 1000] / M_{MIP}$

C_i = initial drug concentration (µmol / mL)

C_f = final drug concentration (µmol / mL)

V_s = volume of solution tested (mL)

M_{MIP} = mass of dried polymer (mg)

Than measuring binding parameter

MIP/drug binding calculated by Scatchard analysis using the equation

$Q / C_f = (Q_{max} - Q) / K_d$

Q_{max} = maximum capacity

K_d = dissociation constant at binding side.

Isotherm adsorption obtained in a thermal water bath at 25 ° C after shaking different concentrations of CPF with a synthesis particle for 2 hours as shown in Figure1.Two and three. Table 4 included experimental data for the regrouping of experiments.

Table 4: Rebinding values of (CPF) using CPF -MIP particles based on (TFMA) and (TMSA)

MAMP-MIP(TFMA)					MAMP-MIP(TMSA)			
Mass of MIP g	Ci mM	C_{free} mM	Q µMole /g	Q/Cfree L/g	Ci mM	C_{free} mM	Q µMole /g	Q/C_{free} L/g
0.1	0.0301	0.0281	0.2	7.117	0.0301	0.0274	0.27	9.8540
	0.0905	0.0785	1.2	15.2866	0.0905	0.0721	1.84	25.5201
	0.1508	0.1281	2.27	17.7205	0.1508	0.1247	2.61	20.9302

	0.2112	0.1982	1.3	6.5590	0.2112	0.1853	2.59	13.9773
0.3	0.0301	0.0221	0.4	18.0995	0.0301	0.0290	0.0366	1.2620
	0.0905	0.0749	0.52	6.9425	0.0905	0.0848	0.19	2.2405
	0.1508	0.1331	0.59	4.432	0.1508	0.1411	0.3233	2.2912
	0.2112	0.1844	0.8933	4.844	0.2112	0.2001	0.37	1.8490

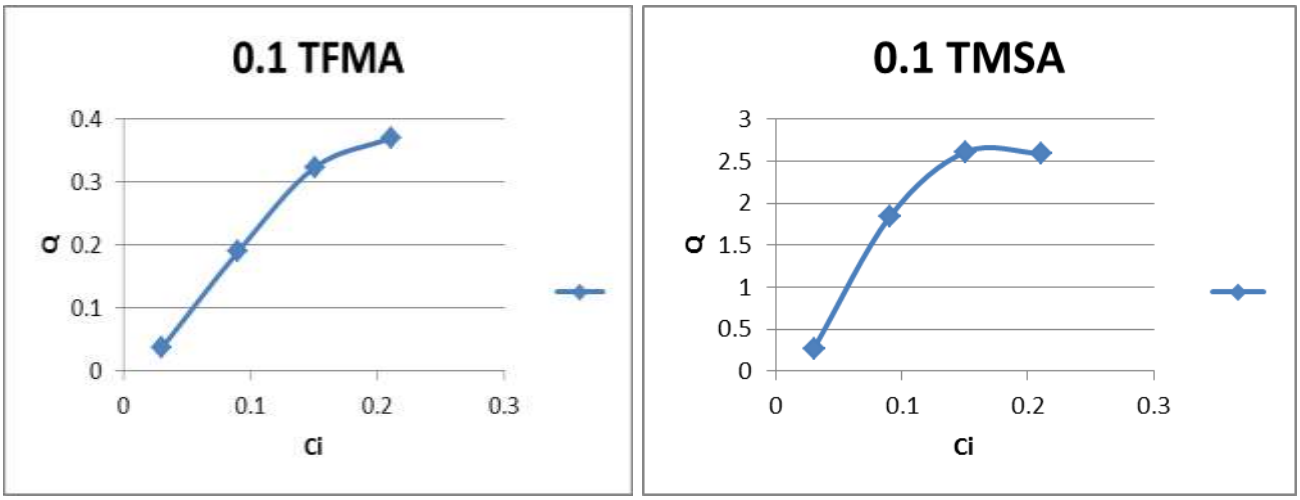
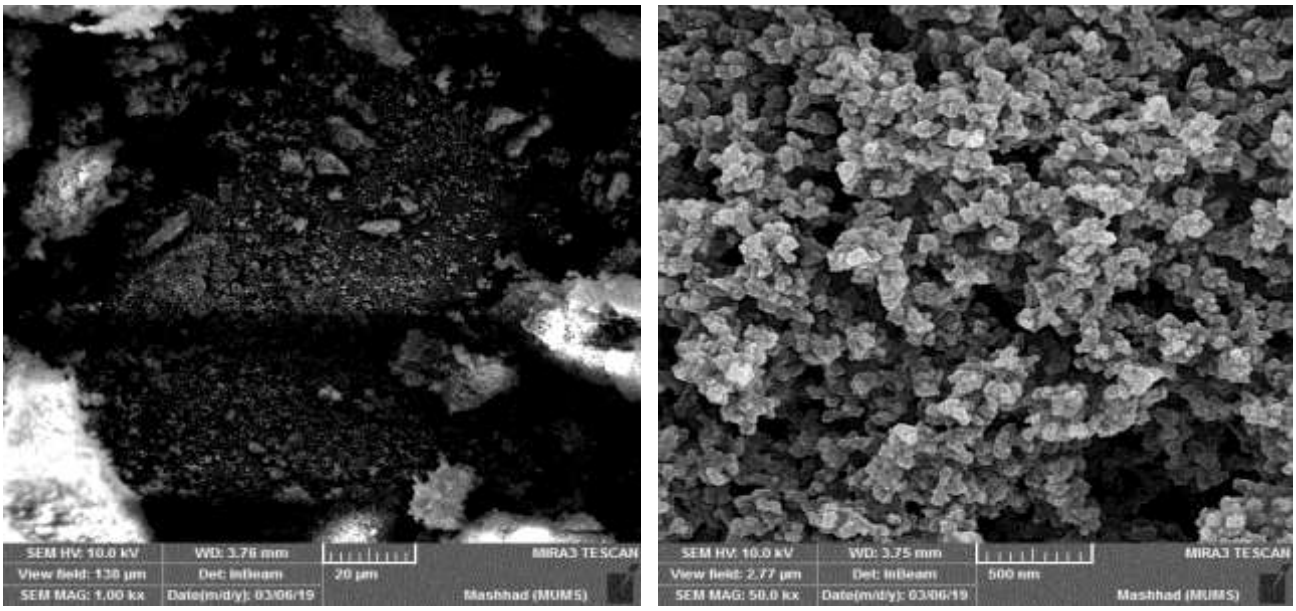


Fig.1: Binding isotherm of TFMA and TMSA monomers by plotting Q against Ci

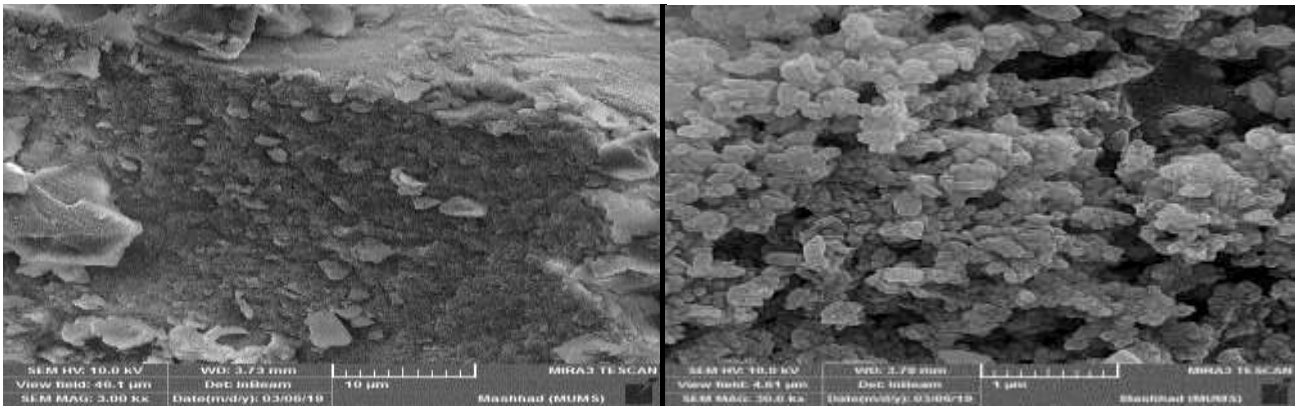
Morphological Characterization

Morphological analysis is an important feature for understanding the size and design of sites that removed MAMP from the polymer.



A **b**

Fig. 2: SEM photograph of the surface of CPF-MIP (TFMA), a) after CPF removal b) before CPF removal



A **b**

Fig. 3: SEM photograph of the surface of CPF –MIP (TMSA), a) after CPF removal b) before CPF removal

Figures 4 and 5 of the SEM images showed that CPF-MIP powders were successfully hybridized into polymer membranes, while the printed membranes show a smooth surface area after the removal of the CPF. Morphological analysis also indicated that the CPF-MIP (TFMA) is more porous than the CPF - MIP (TMSA) structure. The microanalysis shows very small particles and spherically shaped polymeric particles with small sizes around (0.5-1.5) µm for TFMA polymer and (1-3.5) µm for TMSA polymer can be distinguished in the related image figures 2 and 3.

Effect of Flow Rate

The sample solution's flow rate through the manufactured extraction needle is an important factor as it controls the total analytical time and must be sufficient to avoid wasting time. On the one hand, the flow rate must be sufficiently low to effectively retain the analyte. To assess the influence on recovery of the time of contact between the MIP and the sample solution, The effect of the loading rate of the sample was studied in the range of 50 -100 rpm, while the other conditions remained constant. Due to the extraction time and relatively higher extraction efficiency, 70 rpm was selected for further studies as an optimal sample flow rate.

Effect Volume of Sample

Since the separation process takes place using the solid phase extraction technique and depending on the sites in the MIP so that the sample volume affects the separation process. The tube includes 0.1, 0.3 gm of MIP and various volumes of (10 - 70 ppm) analyte solution (1 -10 mL) passed through the tube. After adding 10 mL of CPF, the peak of CPF appearance indicates the saturated MIP when adding volumes greater than 10 mL of CPF. The volumes of less than 10 mL for CPF should, therefore, be selected, displayed good reproducibility ad considered suitable for trace levels determination.

Investigation of Chemical and Thermal Stability

Different experiments showed that manufact ured needles were chemically stable and that the ability to extract manufactured Colum di d not change until 300 ° C capable of extracti ng the temperature limiting application of th ese columns (discussed in detail in ESM).

Samples Analysis

MIP-TFMA and TMSA were used homogenously under optimal conditions to identify ciprofloxacin (CPF) in pharmaceutical samples. The plastic syringe has been taken containing 0.1-0.3 g of MIP (TFMA) and MIP (TMSA) with passing Different concentrations of ciprofloxacin in pharmaceutical samples have been successfully achieved under optimal conditions in a range of 10-70 ppm. Table 5 and 6 show the results.

Table 5: Standard addition method for drug determination using imprinted polymer method solid phase extraction used MIP- TFMA

Wt. of MIP (g)	Conc. of solution (ppm)	Synthetic solution		% Recovery	% RSD
		Conc. Taken (ppm)	Conc. Found (ppm)		
0.1	50	50	49.98	99.96	1.72
0.3	50	50	50.91	101.82	2.51
0.1	30	30	30.73	102.43	2.83
0.3	30	30	30.82	102.73	2.48

Table 6: Standard addition method for drug determination using imprinted polymer method solid phase extraction used MIP- TMSA

Wt. of MIP (g)	Conc. of solution (ppm)	Synthetic solution		% Recovery	% RSD
		Conc. Taken (ppm)	Conc. Found (ppm)		
0.1	50	50	50.28	100.56	1.86
0.3	50	50	49.29	98.58	1.49
0.1	30	30	30.51	101.7	1.12
0.3	30	30	30.95	103.16	2.38

Average of three measurements

The Results obtained from the table 5, 6 found that the proposed method through the preparation of MIP-TFMA and TMSA is acceptable in their standard and pharmaceutical applications.

Conclusion

The research includes the preparation of chemical sensors using various cross-linker monomers to provide the appropriate geometric shape to obtain the molecularly impressed polymers (MIP), as well as knowledge of the drug-prepared capacity of each imprint. On the basis of small concentrations and multiple mixtures, the drug can thus be estimated. Preparation of Ciprofloxacin molecularly impressed polymers, where small percentages of the drug and at different times for the metabolism of the drug can be concentrated and estimated.

The first step was to prepare the MIPs and the second to get my work the process involved the idea of preparing the methamphetamine drug's molecularly impressed polymers capable of estimating small concentrations at different times. The first step was to prepare the molecular printing process and the second to obtain a concentration process Low-dose medicine using solid state extraction, thus obtaining in one step a process per concentration and estimation.

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