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Selective extraction of Ascorbic acid by molecular imprinted polymer solid-phase extraction

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ABSTRACT

A Highly sensitive and selective molecular imprinted polymer (MIP) was synthesized for solid phase extraction and preconcentration of trace amount of Ascorbic acid (AA). Parameters affecting separation and synthesis the polymer such as volume of the solvent, equilibration time, material selectivity and capacity, time, pH, … were checked by ultraviolet (UV) spectroscopy. Then the imprinted polymer applied for extraction of AA with solid-phase extraction, the process done with batch method. The selectivity of method was checked using some real samples. Linear range of the method was examined the data obtained showed linearity over the range of 0.4-9.0 mg/L.

1. Introduction

Vitamin C is an essential nutrient in human and an important water-soluble electron donor in living organisms .Vitamin C has been widely accepted as the most important hydrophilic antioxidant because of having specific and unspecific biological functions and an specific cofactor in enzymatic reactions. Studies have shown that vitamin C deficiency and human mortality are effectively linked [1]. L-Ascorbic acid (AA) (Fig 1) is the main biologically active form of vitamin C. [2]

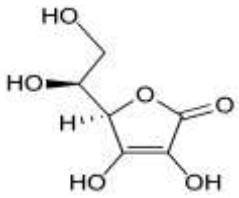

Figure 1. Molecular structures of Ascorbic acid

Although it cannot be syntheses by human, so their main source of the vitamin C is from fruit and vegetables. Therefore, it is very important to carefully study the amount of nutrients in fruits and vegetables and their effect on human health. Some classical and instrumental methods like titrimetry [3], spectrometry [4] and electrochemistry have been used for determination of vitamin C [5]. The best method for determination of AA is separation techniques like liquid chromatography (LC) and Solid phase micro extraction (SMPE) [6-7]. Molecular imprinted polymers [8, 9] have received much attention as a method for preconcentration and initial preparation for measurement methods [10]. Molecularly imprinted polymers (MIPs) are synthetic polymers that synthesised by copolymerizing a monomer with a cross-linker in the presence of a template molecule (print molecule). The template molecule was washed after polymerization .The polymer after washing the template contains sites that was made for template therefore is suitable for target molecule in terms of size, shape and chemical function so the synthesized polymer can perfectly rebind with the template (analyte) and the same molecules. There molecular imprinted polymers could showed highly selective recognition characteristics that is comparable to biological compounds. However, MIPs have several advantages to the biological compounds including, easy preparation, low cast and excellent chemical and physical stability in a wide range of experimental conditions and different solvents. SPME can provide a powerful analytical tool that has the simplicity, application and selectivity of both methods [11]. In this study, the MIP was synthesized for use as sorbent in solid phase extraction of AA. Applicability of the method in real samples was tested and it is found that

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the synthesized polymer is highly selective for AA against expected interferences.

2. Results and Discussion

2.1. Method optimization

2.1.1 Effect of pH

To investigate the effect of pH on the adsorption of polymer. The known equal quantities of the polymer (0.1 gr) and ascorbic acid (5 mg/L) were added to equal volumes (10 ml) of solutions with different pH. After an hour concentration of ascorbic acid in the solutions were measured. The results showed that the maximum adsorption occurred at pH = 6. It seems that in the more alkaline pHs, ascorbic acid is oxidized and in the more acidic pHs H^+ ions compete for binding to polymers with ascorbic acid molecules .then pH = 6 was chosen as optimum pH for this work. Fig 2.

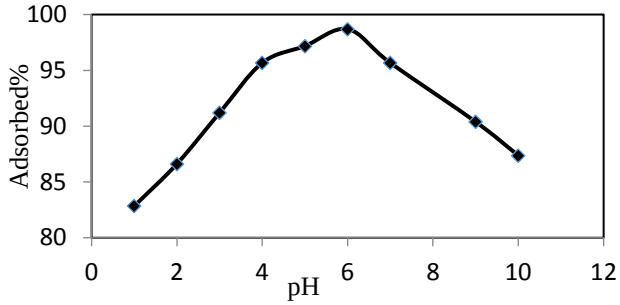


Figure 2. Adsorption on MIP at different pH.

2.1.2. Investigation the effect of stirring time

The effect of stirring time was investigated. The polymer need some time to interact with the analyte and then it will be adsorbed on the polymer .This time is named stirring or equilibrium time. To achieve the optimal stirring time, small and equal amounts of polymer are weighed and placed in contact with ascorbic acid for various times.(10, 20, 30, 40, 50, 60, 75, 80,90and 100 min). The polymers are then filtered, washed and dried and then the AA concentration was measured. The results showed that at first the adsorption of AA increased with increasing time (up to 75 min) , but then with more increase of time it decreased, so we choose 75 min as optimum stir time. (Fig 3)

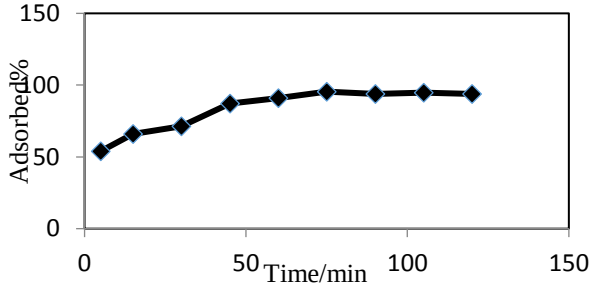


Figure 3. Investigation of effect of stir time for 30 mL acetic acid

2.1.3 Effect of temperature

To investigate the effect of temperature on the adsorption of polymer. The known equal amount of the polymer (0.1 gr) were added to the tubes containing 10 ml solution of ascorbic acid (5 mg/L) at pH=6 .then they placed in water bath with different temperatures. After 75minute concentration of ascorbic acid in the solutions were measured. The results showed that the maximum adsorption occurred at 35° C. Fig 4

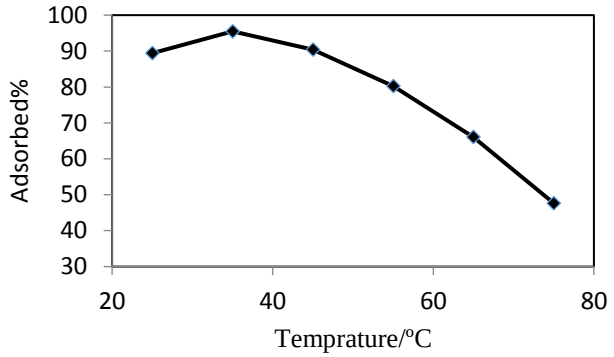


Figure 4. Adsorption capacity of MIP at different temperature at pH=6, t= 75 minute

2.1.4. Effect of solvent volume for maximum desorption of AA

Different volumes of acetic acid (10, 15, 20, 30, 40 and 50 ml) for desorption of AA from polymer was examined. A known amount of polymer (0.10 g) was equilibrated with different volumes of acetic acid for 75 min. The polymers were filtered, washed with distilled water and then dried. Concentration of AA in The acid solutions then measured by spectrophotometric method. From the results obtained it would seem that the best desorption could be done by 30 mL of acetic acid. (Fig 5)

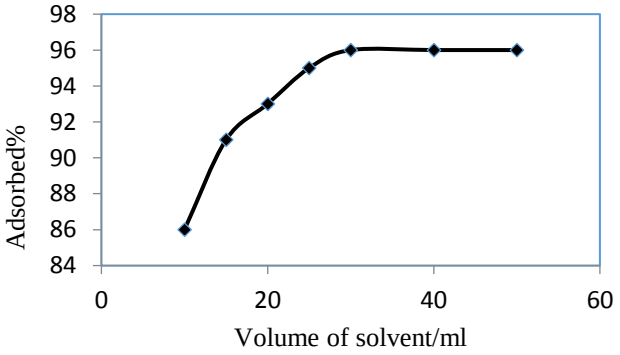


Figure 5. Determination of amount of solvent for desorption of AA with HCl (1M)

2.1.5Effect of polymer weight for maximum adsorption of AA

In order to obtain the maximum absorption at a certain volume of solvent , different amounts of polymer (0.01, 0.015, 0.03, 0.045, 0.060, 0.075 ,0.090 and 0.105 gr) was added to 10 ml of ascorbic acid (5 mg/L) at pH = 6.

The solution was stirred for 75 min. Results showed that the adsorption peak is in 0.075 g of polymer. (Fig 6)

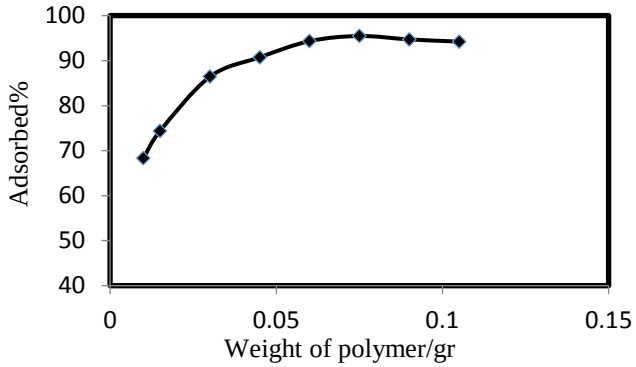


Figure 6. Investigation of amount of polymer on extraction of AA at pH=6.

2.1.6 Investigation of desorption time

To find the optimal washing conditions, the effect of different contact times of wash solution and polymer to separate AA from the polymer surface Checked out. For this purpose, equal amounts of polymer (0.075 gr) are poured into several containers and to each, 30 ml of 0.5 M nitric acid solution was added. The solutions were left for different periods of time (5, 15, 30,45,60,75, 90,105 and 120 minute) meanwhile the solution is stirred by a magnetic stirrer .The polymer was then separated from the solution and the concentration of the solutions was determined. The maximum desorption was observed at 60 minutes and this time was selected as Optimal washing time (Fig 7)

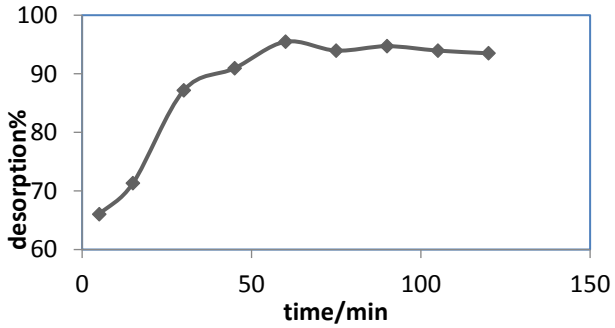


Figure 7. investigation of desorption time

2.2. Selectivity

To estimate selectivity of the synthesized molecularly imprinted polymer, A small and definite amount (0.075 g) of polymer were equilibrated with solution of AA (5 mg/L) in the presence of some biological compound (citric acid, benzoic acid, formic acid , L-aspartic acid , sucrose , fructose and glucose) with over 100 time concentration than AA. The results showed no significant interference and indicate that the imprinted polymer was reasonably selective for AA and so it's applicable in real samples. (Table1)

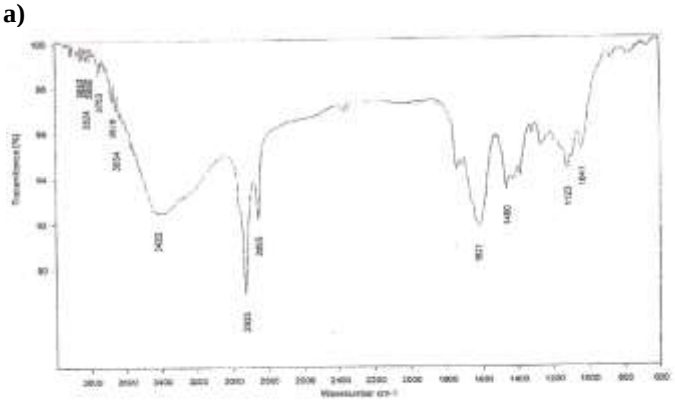
Table 1. Interference study

Analyte	interference	tolerance (mg/L)
Ascorbic acid	citric acid	450
	benzoic acid , formic acid , L-aspartic acid	500
	sucrose , fructose	300
	glucose	350

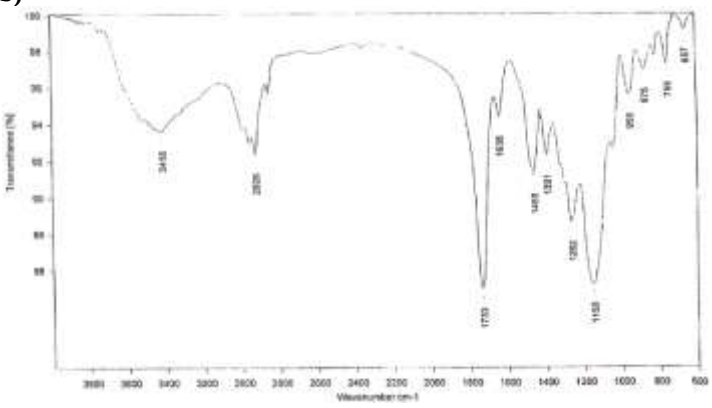
2.3 Characterization of the polymers with FTIR

The MIPs and NIPs, synthesized using chloroform as a porogenic solvent, were characterized by FT-IR spectroscopy. Results are presented in Fig. 8 Comparison of IR spectra of imprinted polymers before and after washing (MIP-Leached, MIP-UnLeached) with IR spectrum of non-imprinted polymer (NIP), indicating that they all have similar spectral shapes, this indicates that the structure is the same for all polymers. The broad absorption bands observed at 3400–3600 cm^{-1} are associated with the intra H bonding in AA. Two significant peaks around 1730 (C=O stretching) and 1145 (C –O stretching) was observed it showed the existence of (EGDMA) as cross linker in the synthesized MIP (16).

a)



b)



c)

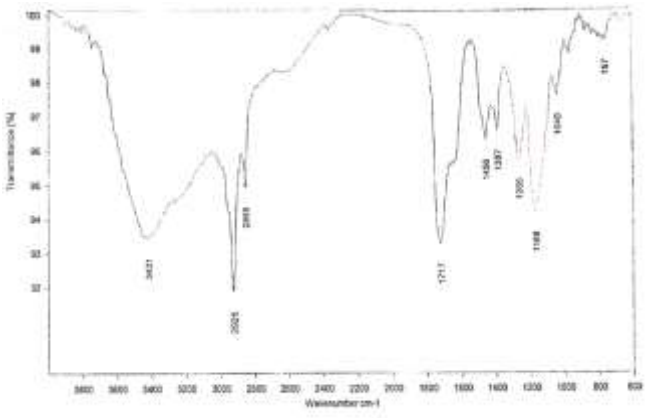


Figure 8. FTIR spectra of leached (a), unleached– MIP (b) and NIP

2.4. Applications of the synthesis polymer

In order to investigate the usability of synthesized polymer for natural samples it was applied it to some fruits juice and the extraction of AA from them with previously mentioned procedure was examined. The results showed good recoveries. (Table 2)

Table 2. Extraction of AA from real samples (n=3)

fruits	Initial concentration ppm	Recovery%
orange	6.64±0.12	94.88%
Kiwi	6.75±0.61	92.73%
tomato	8.71±0.33	93.06%
Melon	7.09±0.30	95.74%
Capsicum	9. 20±0.26	90.15%
citrus	6.18±0.68	96.33%
Tangerine	7.81±0.41	93.23%
Chino	6.18±0.40	95.11%
lemon	8.22±0.21	94.95%
Cucumber	4.07±0.21	97.21%

2.5. Reproducibility of the method

Under optimum conditions (pH =6) AA were extracted and measured by using MIP-SPE To check the reproducibility of the method five replicate experiments were done over the concentration range 0.4-9.0 mg/L .A good R.S.D. (%) was obtained (Table 1). According to the results, accuracy and precision of the method are all satisfactory.

3. Experimental

3.1. General

All chemicals were of analytical-reagent grade and were used without further purification. A buffer and reagent solution was prepared with triply distilled water. Ascorbic acid solution (1000 μg ml^{-1}) was prepared by dissolving appropriate amount of the powder in distilled water fresh daily. Acetic acid (Merck) solution (1 M) was prepared by dilution of stock 37% acid (Merck) with appropriate amount of distilled water. Acetate buffer solution (pH= 6) was prepared according to the literature [12]. Ascorbic acid, acetic acid , potassium iodide , selenium oxide , 7 and 4 buffers , starch glue were prepared from Merck or Fluka companies .N ,N Azobisisobutyronitrile , ethylene glycol dimethacrylate and methacrylic acid all obtained from Merck.

3.2. Apparatus

UV-vis absorption spectra were recorded with a Perkin Elmer spectrophotometer, which is equipped with a 1.00 cm path length quartz cells. pH measurements were made with a pH /Ion meter and a combined glass electrode ,model Metrohm 692 (Metrohm Ltd, CH-9100-Hesau Switzerland). FTIR spectrums were recorded using a Bruker tensor 27 FTIR spectrometer.

3.3 Real Sample preparation

Fruits were obtained from the Shiraz city market. At first about 10 gr of each fruit were divided into small parts. They crushed in a mortar and passed through a filter paper. After that, 5 mL of each extract were diluted with distilled water to 100 mL [13].

3.4. Preparation of molecularly imprinted polymers

Molecularly imprinted polymer was prepared by cross-linking polymerization in the highly diluted solutions. In a glass tube 1 mmol of ascorbic acid (AA), the template, was dissolved in 15 ml of anhydrous acetonitrile. 4 mmol Monomer(methacrylic acid) , 20 mmol ethylene glycol dimethacrylate as cross-linker and initiator(2mmol N ,N Azobisisobutyronitrile) were then added . Dry nitrogen gas was then blown onto the solution for 15 minutes Polymerization can be initiated using UV radiation (350 nm, 20◦C) or temperature (65◦C) In order to complete the polymerization; the glass container was placed in water bath for 24 hours at 65◦C. [14] The polymer was cooled at room temperature and then the polymer was washed with 30 ml acetic acid to remove the template (batch mode). Finally, the synthesized polymer was washed with acetone and dried using vacuum. The non-imprinted polymer was prepared with the same procedure. Only difference was about the template it was omitted from the polymerization process. During all polymerization stages temperature was controlled using a water bath temperature

3.5. Spectrophotometric determination of AA

Determination of AA concentration was done by spectrophotometic method according the literature [15]. At first different amounts of the standard solution containing 0.4 – 9.0 mg /L of AA are added to a series of 10 ml volumetric flasks. Then, the solution was acidified with adding 1.0 mL of 2. 0M hydrochloric acid. After that, a 0.2 mL from a solution of 50 mg /L Se (IV) was added to each flask and wait for 10 min. Then 1.5 mL of KI (0.5 %) was poured to each flask and wait for 2.0 min. then 1.0 mL of starch was poured and the contents of flask mixed well after dilution with distilled water. The solution was set aside for 5 minutes and then the absorbance was measured at 580 nm.The decrease in adsorption is related to the Se (IV) consumed and finally to the AA concentration. Finally, the AA concentration is the result of subtracting the adsorption of the solution containing ascorbic acid and blank solution. The calibration diagram was made by drawing the difference in absorbance against AA concentration. AA concentration was obtained from the calibration curve.

4. Conclusion

Solid phase extraction is a popular sample preparation method and although it is not a new method, it is still considered and applied. Although, like any other method, it has changed a lot over time.a lot of compounds have been used as solid phase one of them are polymers. Although polymers have already used as solid phase, the use of Molecular imprinted polymers allows it used as a specific compound. In this manuscript, a simple and novel procedure was applied to synthesize AA-imprinted polymer using molecular imprinting technique. After the polymer, synthesized parameters affecting absorption of ascorbic acid molecules were investigated. The parameters were pH, equilibrium adsorption time, amount of polymer, and optimum concentration of extraction and volume solvent etc. Then amount of ascorbic acid extracted was determinate using spectrophotometric method. The results show that imprinting of the polymer causes selectivity and high absorption of AA on the polymer. Solid-phase extraction and determine AA was done by the synthesized polymer for some real samples. The results showed good recoveries for MIP–SPE method also high selectivity were obtained. The accuracy and precision of the method are obtained and all were satisfactory.

Acknowledgements

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