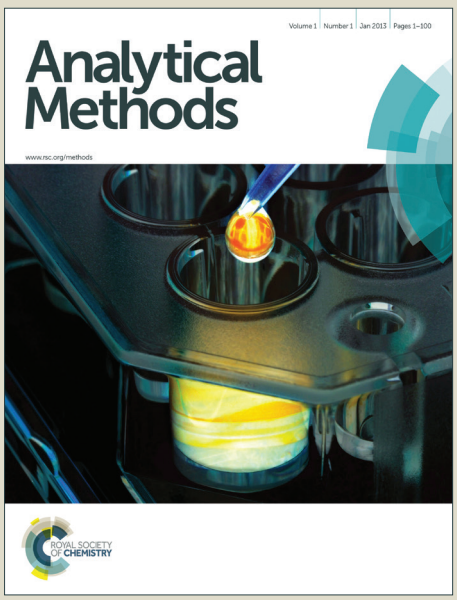
Analytical Methods

Accepted Manuscript



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Synthesis and comparison of new layer-coated silica nanoparticles and bulky molecularly imprinted polymers for the solid-phase extraction of glycine

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Abstract: Imprinted polymers were prepared using bulky and layer-coated silica nanoparticles to analyze trace glycine in human urine. In the layer-coated silica nanoparticle-imprinted polymer, the polymerizable double bonds were first grafted on the surface of silica nanoparticles throgh silylation to induce the selective occurrence of surface polymerization. Then, glycine templates were imprinted into the polymer-coated layer through interaction with functional monomers. The molecularly imprinted polymer (MIP) and SiO_2-MIP were tested in batch experiments to evaluate their binding properties and then used as SPE sorbents for the selective removal and pre-concentration of glycine. The glycine-imprinted polymer nanoparticles presented higher selectivity and affinity to glycine than bulky imprinted polymer. Glycine was directly extracted from spiked human urine. MIP and SiO_2-MIP allowed glycine to be pre-concentrated while removing interfering compounds from the matrix. SiO_2-MIP showed high efficiency for the enrichment of glycine in real samples.

Keywords: Glycine; Molecularly imprinted polymer; Layer-coated silica nanoparticles; Solid-phase extraction

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Introduction

In the last decade, molecularly imprinted polymers (MIPs), have attracted considerable attention as synthetic antibody mimics because of their outstanding advantages, such as high selectivity and affinity to the target molecule, high mechanical strength, chemical stability, and reusability[1-4]. These properties provide board opportunities for the use of MIPs in numeuros fields. However, the practical applications of MIPs are limited by the difficult separation of small particles from aqueous samples [5].

Traditional methods to prepare MIPs involve bulk/precipitation polymerization and yield bulky MIPs, most often result in materials exhibiting high affinity and selectivity but suffer from poor site accessibility, incomplete template removal, small binding capacity, slow mass transfer, and irregular material shape[6].

Attempts to address these problems generally require imprinted materials to be prepared by using optimizing forms that control templates to be situated on the surface or near the material surface[7].

Grafting can be used for molecular imprinting on the surface of polymer/silica beads and the resulting MIP composites have the advantages of more accessible binding sites and faster mass transfer compared to the MIPs prepared by conventional bulk polymerization techniques. [8-11]. For instance, MIPs have been prepared as a grafted coating on silica particles [12-17], silica capillary columns [18], Fe_3O_4 magnetic nanoparticles [5, 19, 20],alumina oxide membrane [21], and polymeric supports [22, 23].

The present work describes the synthesis and comparison of bulky and layer-coated silica nanoparticle MIPs as a highly selective sorbent for the solid-phase extraction (SPE) of glycine. Glycine is a fundamental amino acid. Mutations that lead to the replacement of glycine by other amino acids may result in the malfunction of certain proteins and lead to diseases such as

osteogenesis imperfecta and Ehlers–Danlos syndrome [24].

The prominent function of glycine in living creatures warrants an accurate and precise quantitative analysis method for the compound. Various methods for measuring glycine and other amino acids have been reported. For glycine measurement in biological fluids, amino acids are usually separated first through high-performance liquid chromatography with precolumn or postcolumn derivatization, and then the derivatized analyte is detected using UV [25], fluorescence[26], or MS[24] . These methods are accurate but expensive, and analysis can be laborious. However, the routine determination of glycine in large sets of clinical samples requires simple and inexpensive methods.

Conventional SPE materials, such as C18, are nonpolar and nonselective, making them unsuitable for the extraction of polar compounds such as glycine. In the present work, the efficacy of the prepared MIP and SiO_2–MIP was evaluated and compared for glycine adsorption. Finally, glycine–MIP and SiO_2–glycine–MIP were successfully applied for the SPE of glycine in urine samples.

Experimental

Instrumentation

A UV-Vis spectrophotometer (Cary 100, Varian, Australia) was used to measure glycine in standard solutions after contact with the polymers. A Soxhlet extraction apparatus was used to remove the target molecule of the polymer network. A model 744A Metrohm pH meter was used to adjust pH. The Fourier transform infrared (FTIR) spectra of the nonimprinted polymer (NIP), and MIP were obtained using a 6700 Thermo Nicolet FTIR spectrometer at 400–4000 cm^{-1}.

The powder X-Ray diffraction (XRD) patterns of SiO_2–MIP and silica nanoparticles were obtained using a powder diffractometer (Bruker-D8) with Cu Kα radiation. The accelerating voltage and current used were 40 kV and 20 mA, respectively. Scanning electron microscopy (SEM) was performed by gently distributing the powder sample onto stainless steel stubs and using a SEM (Philips, XL30, Almelo, the Netherlands) instrument. Thermogravimetric analysis (TGA) was carried out by using TGA-50H Shimadzu (Kyoto, Japan).

Reagents and standards

All chemicals and reagents were of analytical grade and used without any further purification. Methacrylic acid (MAA), 2,2'-azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA), tetraethylosilicate (TEOS), 3-(methacryloxy)propyltrimethoxysilane (MPTS), ninhydrin, chloroform, methanol, hydrochloric acid, sodium hydroxide, sulfuric acid, ethanol, acetic acid, potassium hydroxide, potassium dihydrogen phosphate, phosphoric acid, and acetonitrile were obtained from Merck. Glycine, sarcosine, alanine, valine, and lysine were obtained from Sigma–Aldrich.

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All experiments were performed in compliance with the relevant laws and institutional guidelines, and This project was approved by research committee of Damghan Brnch, Islamic Azad University.

Synthesis of bulky MIPs

The polymer imprinted with glycine (MIP) was prepared as follows. Glycine (1 mmol) was dissolved in a water/acetonitrile solution (4/1, v/v; 10 mL) in a glass tube, to which 4 mmol of MAA and 16 mmol of EGDMA were added. The mixture was added with 0.084 mmol of AIBN, degassed in a sonicating bath, flushed with nitrogen gas for 5 min to remove oxygen, sealed, and then incubated at 60 °C for 20 h to polymerize.

The resulting polymer was ground in a mortar, sieved, and then washed several times with methanol/acetic acid (7/3, v/v) and then with methanol to remove residual acetic acid.

The particles were vacuum dried and then used for rebinding studies and preparing SPE cartridges. A control NIP was prepared using the same conditions but without the addition of the template (glycine).

Synthesis and chemical modification of silica nanoparticles

Monodispersed spherical silica particles were prepared through the hydrolysis of TEOS [27]. Solutions I (5 mL of TEOS in 30 mL of ethanol) and II (9 mL of ammonia in 50 mL of ethanol) were prepared separately.

Solution I was added into a round-bottom flask containing solution II by using a micro-feed pump at 0.025 mL min^{-1} flow rate and room temperature under vigorous stirring at 750 rpm. The mixture was allowed to react for 24 h after addition. The resulting silica nanoparticles were separated through centrifugation at 10,000 rpm for 10 min and then washed with ethanol to remove residual ammonia. Subsequently, the monodispersed silica nanoparticles were chemically modified with MPTS to obtain polymerizable double bonds. Following a

conventional method, 0.1 g of silica nanoparticles and 2 mL of MPTS were added into toluene to prepare 20 mL of mixed solution. The mixture was refluxed for 12 h under high-purity nitrogen. The resulting MPTS–silica nanoparticles were separated via centrifugation and then washed with toluene.

Imprinting of glycine molecules on the surface of MPTS–Silica

Prior to polymerization, a solution was prepared by dissolving glycine (1 mmol) and MAA (4 mmol) in 25 mL of acetonitrile and then stored in the dark for 12 h. MPTS–silica nanoparticles (0.16 g) were dispersed in 25 mL of toluene–acetonitrile (4/1, v/v) through ultrasonic vibration. The prearranged solution, EGDMA (16 mmol), and AIBN (0.16 mmol) were then dissolved into the above solution. The mixed solution was purged with high-purity nitrogen for 10 min while cooling in an ice bath.

A three-step temperature polymerization reaction was carried out in an incubating shaker at 300 rpm. Prepolymerization was first conducted at 50 °C for 6 h, and polymerization was completed at 60 °C for 24 h. Subsequently, the temperature was raised from 60 °C to 75 °C in 1 h at 0.25 °C min^{-1}, and the products were further aged for 6 h at 75 °C to obtain high cross-linking density. The resulting SiO_2–glycine–MIP nanoparticles were separated from the mixed solution through centrifugation. The nonimprinted polymer (SiO_2–NIP) nanoparticles were also prepared as described above but without the addition of the template. Finally, the obtained nanoparticles were ultrasonically cleaned with methanol–acetic acid (9/1, v/v) to remove the template, washed with methanol, and then vacuum dried at room temperature.

Determination of glycine by ninhydrin

A 1 mL portion of the reaction mixture consisting of citrate buffer (0.35 mol L^{-1}), ninhydrin (5 mg), and glycerol (6/4 v/v) was pipetted into a test tube. A 1 mL sample was introduced to the reaction mixture. After shaking, the test tube was placed in a boiling water bath for 10 min. The test tube was cooled, and its absorbance was mesured at 570 nm against a reagent blank prepared in the same manner [28].

Batch rebinding experiments

A buffer solution, the glycine solution, and the immersed imprinted polymer were added into 50 mL polyethylene bottles with shaking at 25 °C. At a preset time, an aliquot of the supernatant was separated, and glycine was determined spectrophotometrically in accordance with the aforementioned ninhydrin method at 570 nm. The adsorbed glycine was eluted with water/ethanol (5/5, v/v), and the desorbed glycine was measured as previously described.

SPE cartridge experiments

A 200 mg sample of MIP was packed dry in an empty SPE cartridge between two polyethylene frits. The cartridge was activated by 5 mL of acetonitrile and then conditioned by 5 mL of water. An aliquot (5 mL) of a 1 mg L^{-1} water solution of glycine was loaded on the cartridge at a flow rate of 1 mL min^{-1}. Afterward, the cartridge was first washed with 1 mL of acetonitrile to elute unbound compounds, and then glycine was eluted with 2 mL of water/ethanol (5/5, v/v). A flow rate of 1 mL min^{-1} was used in both washing and elution steps. The eluted glycine was determined spectrophotometrically by using the ninhydrin method at 570 nm.

The phase distribution ratio (K_d) and adsorption capacity (Q) were calculated using the following equations: :

$$Kd = \frac{C_i - C_f}{C_f} \times \frac{V}{W} \quad (1)$$

$$Q = \frac{(C_i - C_f)V}{w} \quad (2)$$

where Q represents the adsorption capacity (μmol g^{-1}); Ci and C_f represent the initial and equilibrium concentrations of glycine in the aqueous phase (μmol L^{-1}), respectively; W is the weight of the polymer (g); and V is the volume of the aqueous phase (L). The extraction percentage E was calculated using the following equation:

$$E = \frac{C_i - C_f}{C_i} \times 100 \quad (3)$$

Interference effects

A 2 mL aliquot of a 10 mg mL^{-1} water solution of each amino acid (glycine, sarcosine, alanine, valine, and lysine) was loaded on the MIP–SPE and SiO_2–MIP–SPE cartridges. Then, the cartridges were washed with 1 mL of acetonitrile to elute unbound compounds and to increase the selective interaction. Finally, the amino acids were eluted with 2 mL of water/ethanol (5/5, v/v). The collected amino acids were carefully analyzed spectrophometrically by using the ninhydrin method. In addition, a 2 mL aliquot of a 10 mg mL^{-1} water solution of a binary mixture of these amino acids and glycine was loaded on the MIP–SPE and SiO_2–MIP–SPE cartridges.

Extraction of glycine from spiked human urine

The MIP–SPE and SiO_2–MIP–SPE cartridges prepared above were activated by 5 mL of acetonitrile and then conditioned by 5 mL of water.

An aliquot (2 mL) of human urine spiked with glycine (5 and 10 mg L^{-1}) was loaded on the cartridges at a flow rate of 1 mL min^{-1}. The cartridges were first washed with 1 mL of acetonitrile, and then glycine was eluted with 2 mL of water/ethanol (5/5, v/v). A flow rate of 1 mL min^{-1} was used in both washing and elution steps. All eluted fractions were collected, and the eluted glycine was determined spectrophotometrically by using the ninhydrin method at 570 nm.

Results and discussion

Bulky and layer-coated silica nanoparticle MIPs were prepared for glycine. The structures were examined via FTIR spectroscopy, XRD, and SEM.

The adsorption time, adsorption capacity, and effect of pH were evaluated and compared between the bulky and layer-coated silica nanoparticle MIPs.

Characterization of synthesized polymers

FTIR spectroscopy was performed for the bulky and layer-coated silica nanoparticle MIPs. Both polymers presented similar IR spectra, indicating similarity in the backbone structure. The band at approximately 470 cm^{-1} resulted from Si–O vibrations. The Si–O–Si band around 1100 cm^{-1} overlapped with the C−O band. Absorbance values that were attributed to the methyl (or methylene) groups at 2800–3000 cm^{-1} for the layer-coated silica nanoparticle MIPs corresponded to the stretching vibration of C–H bonds and were relatively stronger than those for

the bulkyimprinted polymer. In addition, the IR spectra of MIPs and NIPs present nearly identical characteristic peaks.

The FTIR spectra of methacrylic acid, ethylene glycol dimethacrylate, MIP and SiO_2–MIP are shown in Figure 1 and 2.

Fig. 1. FT IR spectra of (A) methacrylic acid and (B) ethylene glycol dimethacrylate

Fig. 2. FTIR spectra of MIP (a) and SiO_2–MIP

The structural properties of SiO_2 nanoparticles and layer-coated silica imprinted polymer were analysized by X-ray power diffraction (XRD).

Figure 3 shows the XRD spectra of SiO_2 nanoparticles and layer-coated silica imprinted polymer. XRD patterns of the synthesized SiO_2 nanoparticles and layer-coated silica imprinted polymer display several reflectionpeaks in the 2θ region of 20°–70°.

The peak at $2\theta = 27°$ is the main peak of crystalline silica and is also present in the SiO_2–MIP spectra.

Fig. 3. XRD patterns of SiO_2 nanoparticles (a) and SiO_2–MIP (b).

The morphologies of SiO_2–MIP and MIP were assessed through SEM. The SEM patterns (Figure 4) show the formation of SiO_2–MIP nanoparticles in comparison with that of bulky MIPs.

Fig. 4. SEM images of MIP (a) and SiO_2–MIP (b).

The thermal decomposition pattern of silica and layer-coated silica nanoparticle MIP were showed in Figure 5. Silica had only one weight loss stage around 100 °C corresponding to the release of physically adsorbed water. layer-coated silica nanoparticle had two weight loss stage: First stage around 280 ~ 320 °C, which is rapid, corresponding to the degradation MIP, and the second stage (slow weight loss around 320 ~ 600 °C) could be corresponded to the decomposition of char formed in the previous stage [29]. the degradation of MIP, and the loss weight (39%) indicating that MIP grown on the surface of silica gel.

Fig. 5 shows the thermal decomposition pattern of (A) silica and (B) layer-coated silica nanoparticle

Optimization of adsorption conditions of glycine on polymer

Effect of time and flow rate on the adsorption of glycine

Six portions of standard or sample solutions (25 mL) containing glycine (0.125 mg) were transferred into 50 mL beakers. Then, 0.2 g of MIP and SiO_2–MIP adsorbents were added to each beaker, and the mixtures were shaken vigorously for 30, 60, 90, 120, 150, and 180 min to facilitate adsorption of glycine onto the imprinted polymer particles. After the solutions were centrifuged, the amount of unadsorbed glycine in the filtrate solutions was determined spectrophotometrically. Figure 6 shows that approximately 90% sorption of glycine was achieved in equilibrium times of 150 and 90 min for MIP and SiO_2–MIP, respectively. The amount of glycine bound to the polymer was calculated by subtracting the amount of unadsorbed substrate from the initial amount of template. Glycine was adsorbed on SiO_2–MIP within significantly shorter times rather than the bulky imprinted polymer. In addition, the effect of flow

rate was optimized at 0.5–2 mL min^{-1} in the SPE experiment. The maximum adsorption was achieved at flow rates exceeding 1 mL min^{-1}. Therefore, the flow rate of 1 mL min^{-1} was selected for further experiments.

Fig. 6. Influence of adsorption time on the extraction of glycine (MIP and SiO_2–MIP)

Effect of sample pH on glycine adsorption

The effect of various pH values on glycine uptake was investigated using a batch procedure. Six portions of standard or sample solutions (25 mL) containing glycine (0.125 mg) were transferred into 50 mL beakers, and the pH was adjusted to 3–9 by using 0.01 mol L^{-1} HNO_3 or NaOH. Exactly 0.2 g of the adsorbent was added to each beaker, and the mixtures were shaken vigorously for 150 and 90 min for MIP and SiO_2–MIP, respectively. As shown in Figure 7, the adsorption quantity of glycine increased with pH, and the maximum adsorption occurred at pH 7.0.

Therefore, pH 7.0 was selected for this experiment because the adsorption capacity of the polymer decreases beyond this pH level. SiO_2–MIP was sensitive to pH.

Fig. 7. Effect of pH of sample solution on glycine uptake (MIP and SiO_2–MIP)

Adsorption capacity of glycine by MIP

The adsorption of glycine from the sample solution was investigated in batch experiments. At this stage, the effect of sample concentration on glycine adsorption was investigated to obtain the best concentration for the sample solution. Solutions with concentrations of 10^{-5}, 10^{-4}, 10^{-3}, and

10^{-2} mmol L^{-1} glycine were prepared, and the pH was adjusted to 7.0 by using 0.01 mol L^{-1} HNO_3 or NaOH. Exactly 0.2 g of the adsorbent was added to each beaker, and the mixtures were shaken vigorously for 150 and 90 min for MIP and SiO_2–MIP, respectively. To reach saturation, the initial glycine concentrations were increased until plateau values (adsorption capacity values) were obtained. The data are shown in Figure 8. The average maximum adsorption capacities were 1.04×10^{-3} and 1.35×10^{-3} mmol L^{-1} for MIP and SiO_2–MIP, respectively. The adsorption capacity increased by 35% through the layer-coated silica nanoparticle MIPs rather than the bulky MIPs.

Fig. 8. Effect of initial glycine concentration on the adsorption quantity of MIP and SiO_2–MIP. Other conditions: 0.2 g of synthesized polymer; pH 7.0; shaking time, 150 and 90 min for MIP and SiO_2–MIP, respectively; and temperature, 25 °C.

Comparison of MIP and NIP adsorption

Four solutions were prepared with 10^{-4} mol L^{-1} glycine, and the pH was adjusted to 7.0 by using 0.01 mol L^{-1} HNO_3 or NaOH. Then, 0.2 g of MIP was added to one solution, whereas 0.2 g each of NIP, SiO_2–MIP, and SiO_2–NIP were added to the others. The mixtures were shaken vigorously for 150 and 90 min for MIP and SiO_2–MIP, respectively. All filtrate and glycine concentrations in the solutions were measured. Table 1 shows that SiO_2–MIP performed better adsorption than MIP and NIPs, and these results confirm the accuracy of the molecular format.

Table 1. Comparison of MIP, NIP, SiO_2–MIP, and SiO_2–NIP.

Efficient eluent

To select a proper eluent for the retained glycine, glycine was stripped using 5 mL of various concentrations of different organic and mineral acids after the extraction of 0.025 mmol glycine from 25 mL of the aqueous sample solution. To select the most efficient eluent, different organic solvents and various concentrations of different acids in organic solvents were tested. As shown in Table 2, polar eluents are more effective in stripping glycine from the polymer.

On the basis of the data given in Table 2, 5 mL of water/ethanol (5/5, v/v) can strip the retained glycine almost quantitatively. Thus, this eluting solvent was selected for further studies.

Table 2. Effect of eluent type on extraction efficiency

Interference effects

After evaluating the efficiency of MIP and SiO_2–MIP, the selectivity of the polymers in cartridge experiments were investigated. In particular, the performance of MIP as the sorbent for the SPE of glycine was evaluated and compared with that of SiO_2–MIP.

Table 3 shows the recovery yields in the elution solution after the extraction of the amino acids using MIP and SiO_2–MIP cartridges. Extraction recovery yields were 87.2% for MIP and 93.7% for SiO_2–MIP. The SiO_2–MIP under the same conditions allowed the major portion of glycine to elute in the loading and washing steps.

Table 3. Recovery yields in the elution solution after the extraction of amino acids using MIP and SiO_2–MIP cartridges.

Table 4 shows the elution profile obtained for MIP and SiO_2–MIP cartridges when a binary mixture was loaded. Glycine was eluted during the elution step with a recovery yield of 85.4%–74.2% and 91.2%–86.3% for MIP and SiO_2–MIP, respectively. These data confirmed the possibility of washing interfering compounds from the MIP while retaining the analyte and emphasized the higher selectivity of SiO_2–MIP toward glycine.

Table 4. Recovery yields in the elution solution after the extraction of the binary mixture using the MIP cartridge.

Analytical approach

Under optimum conditions, calibration curves were obtained for glycine by the bulky and layer-coated silica nanoparticle MIPs in spiked water solutions.

In spiked water solutions, calibration standards were prepared at concentrations of 0.1, 0.2, 0.4, 0.8, 1, 2, 4, 5, 8, and 10 mg L^{-1}. Good linearity for glycine was observed in the entire range of tested concentrations, as proven by the correlation coefficients. R^2 was greater than 0.9724 for the two synthesized polymers (figure 9). A detection limit of 0.01 mg mL^{-1} was achieved through preconcentration of 10 mL sample solution. The detection limit can be enhanced through

analyte preconcentration from a large volume of sample solution because of the complete adsorption of glycine onto the MIPs. The relative standard deviations for glycine were 5.4% and 5.2% for the bulky and layer-coated silica nanoparticle MIPs, respectively.

Fig. 9. Calibracutin curve of glycine

Extraction of glycine from spiked human urine

Glycine in urine samples was separated and preconcentrated by synthesized MIP and SiO_2–MIP. As shown in Table 5, glycine was extracted (thus retained) by MIP and SiO_2–MIP, indicating that glycine was preconcentrated in presence of interfering compounds. SiO_2–MIP was highly efficient for the enrichment and removal of glycine in real samples.

Table 5. Determination of glycine in human urine

Conclusion

In this study, bulky and layer-coated silica nanoparticle glycine imprinted polymers were prepared. The resulting SiO_2–glycine–MIP nanoparticles exhibited superior spherical and uniform morphology and higher glycine selectivity. Compared with the traditional bulky method, the combination of imprinted layer-coated nanostructures with the surface enrichment of targets can significantly improve the binding capacity and kinetics of imprinted materials by increasing the amount of binding sites on the surface or near the material surface. In addition, MIPs were

successfully used in SPE to selectively enrich and determine glycine from spiked urine samples. The use of SiO_2_glycine–MIP or even glycine–MIP in SPE as an alternative method to other techniques of glycine separation and preconcentration offers several advantages, including low cost, high capacity with high recovery, and excellent extraction efficiency. This method provides a selective, simple, and practical strategy for glycine determination.

Acknowledgments

We are grateful to the Laboratory Complex of Damghan Branch, Islamic Azad University and Pars Material Research and Testing (PMRT) for valuable technical assistance for valuable technical assistance.

References

1. H. Hashemi-Moghaddam and M. Shakeri, *Korean Journal of Chemical Engineering*, 2014, 1-5.
2. B. Sellergren, *Analytical chemistry*, 1994, 66, 1578-1582.
3. Y. Li, H.-H. Yang, Q.-H. You, Z.-X. Zhuang and X.-R. Wang, *Analytical chemistry*, 2006, 78, 317-320.
4. H. Hashemi-Moghaddam and M. R. Alaeian, *Journal of the Chinese Chemical Society*, 2014.

5. L. Chang, S. Chen and X. Li, *Appl. Surf. Sci.*, 2012, 258, 6660–6664.

6. H. Hashemi-Moghaddam, M. Rahimian and B. Niromand, *Bull. Korean Chem. Soc*, 2013, 34, 2331.

7. Y. Peng, Y. Xie, J. Luo, L. Nie, Y. Chen, L. Chen, S. Du and Z. Zhang, *Anal. chim. acta*, 2010, 674, 190-200.

8. M. Glad, P. Reinholdsson and K. Mosbach, *React. Polym.*, 1995, 25, 47-54.

9. O. Norrlöw, M. Glad and K. Mosbach, *J. Chromatogr. A*, 1984, 299, 29-41.

10. S. D. Plunkett and F. H. Arnold, *J. Chromatogr. A*, 1995, 708, 19-29.

11. Y. Lv, T. Tan and F. Svec, *Biotechnol. Adv.*, 2013, In press.

12. C. Zhai, Q. Lu, X. Chen, Y. Peng, L. Chen and S. Du, *J. Chromatogr. A*, 2009, 1216, 2254-2262.

13. H. He, G. Fu, Y. Wang, Z. Chai, Y. Jiang and Z. Chen, *Biosens. Bioelectron.*, 2010, 26, 760-765.

14. W. Zhao, N. Sheng, R. Zhu, F. Wei, Z. Cai, M. Zhai, S. Du and Q. Hu, *J. hazard. mater.*, 2010, 179, 223-229.

15. R. Gao, J. Zhang, X. He, L. Chen and Y. Zhang, *Anal. Bioanal. Chem.*, 2010, 398, 451-461.

16. Y.-K. Lv, Y. Ma, X.-B. Zhao, C.-L. Jia and H.-W. Sun, *Talanta*, 2012, 89, 270-275.

17. Q. Lu, X. Chen, L. Nie, J. Luo, H. Jiang, L. Chen, Q. Hu, S. Du and Z. Zhang, *Talanta*, 2010, 81, 959-966.

18. L. Schweitz, *Anal. chem.*, 2002, 74, 1192-1196.

19. X. Wang, L. Wang, X. He, Y. Zhang and L. Chen, *Talanta*, 2009, 78, 327-332.

20. X. Kong, R. Gao, X. He, L. Chen and Y. Zhang, *J. Chromatogr. A*, 2012.

21. H.-J. Wang, W.-H. Zhou, X.-F. Yin, Z.-X. Zhuang, H.-H. Yang and X.-R. Wang, *J. Am. Chem. Soc.*, 2006, 128, 15954-15955.

22. M. Ulbricht, *J. chromatogr. B*, 2004, 804, 113-125.

23. A. Bossi, S. A. Piletsky, E. V. Piletska, P. G. Righetti and A. P. Turner, *Anal. chem.*, 2001, 73, 5281-5286.

24. Y.-B. Tang, L. Teng, F. Sun, X.-L. Wang, L. Peng, Y.-Y. Cui, J.-J. Hu, X. Luan, L. Zhu and H.-Z. Chen, *Journal of Chromatography B*, 2012, 905, 61-66.

25. H. Bevers, R. Wijntje and A. De Haan, 2009.

26. T. P. Piepponen and A. Skujins, *Journal of Chromatography B: Biomedical Sciences and Applications*, 2001, 757, 277-283.

27. K. Nozawa, H. Gailhanou, L. Raison, P. Panizza, H. Ushiki, E. Sellier, J. Delville and M. Delville, *Langmuir*, 2005, 21, 1516-1523.

28. Y. P. Lee and T. Takahashi, *Analytical biochemistry*, 1966, 14, 71-77.

29. W. Cheng, Z. Liu and Y. Wang, *Talanta*, 2013, 116, 396-402.

Figures Captions:

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Fig. 2. FT IR spectra of MIP (a) and SiO_2-MIP

Fig. 3. XRD patterns of SiO_2 nanoparticles (a) and SiO_2–MIP (b).

Fig. 4. SEM images of MIP (a) and SiO_2–MIP (b).

Fig. 5 Thermal decomposition pattern of silica and layer-coated silica nanoparticle

Fig. 6. Influence of adsorption time on the extraction of glycine (MIP and SiO_2–MIP)

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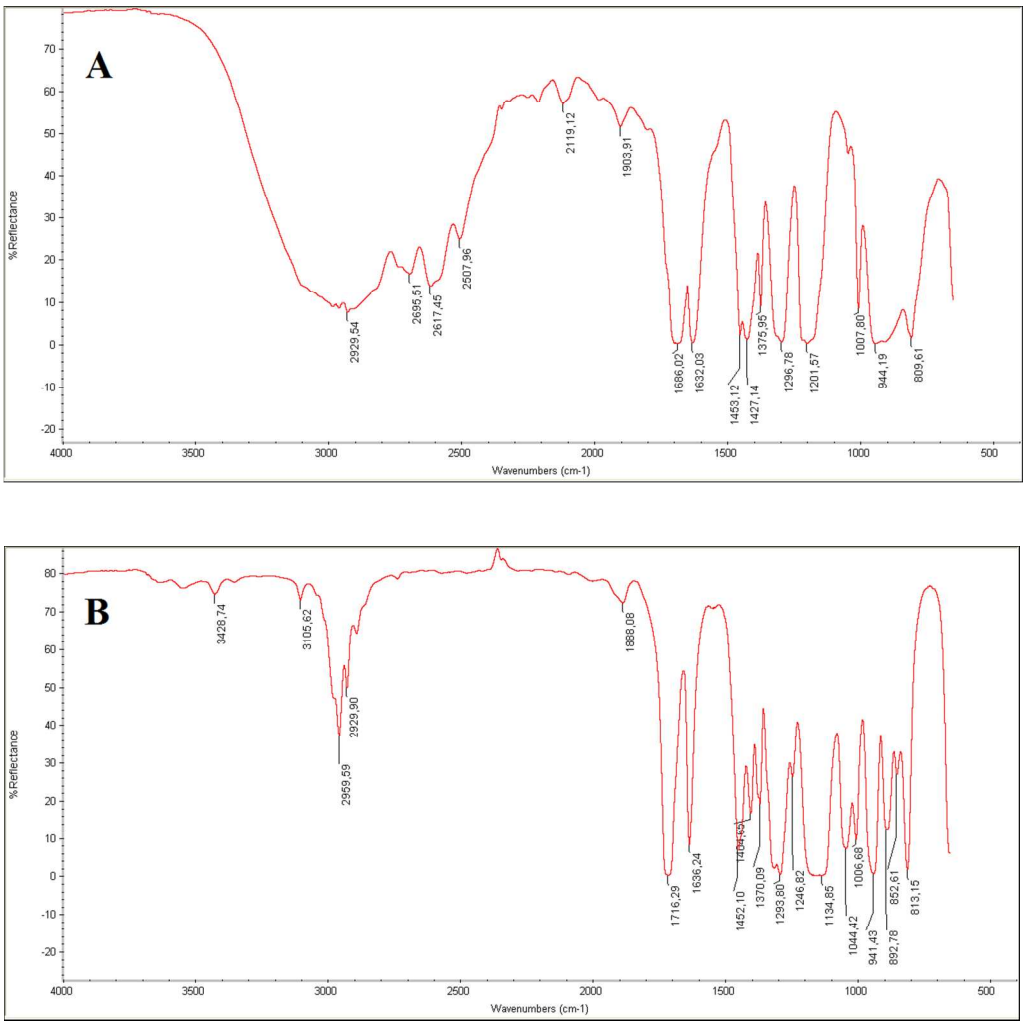

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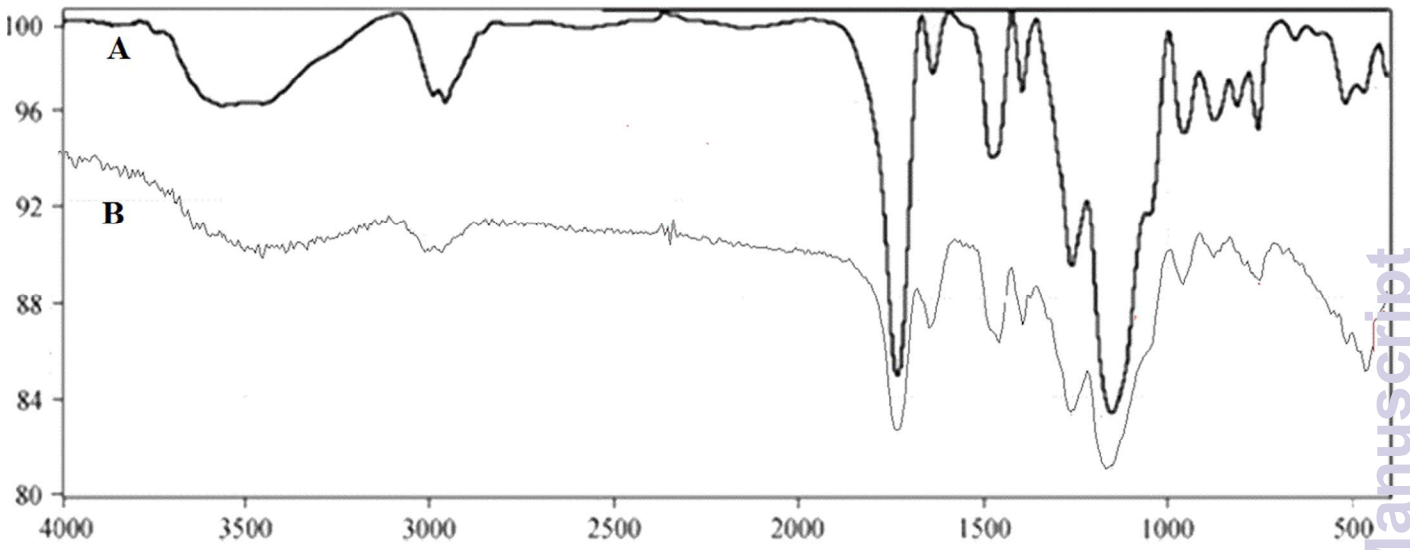
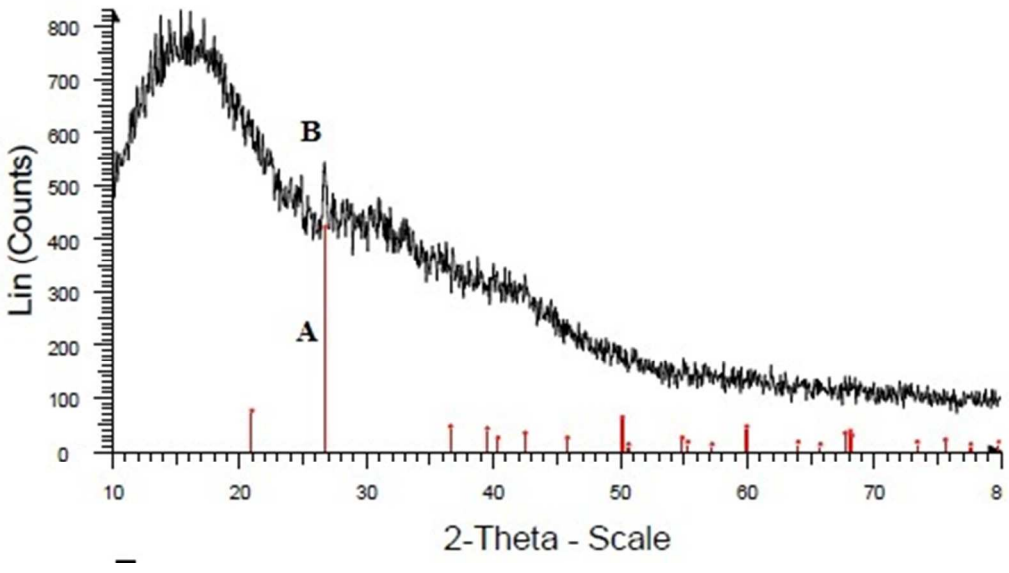


Fig. 2. FT IR spectra of (A) MIP and (B) SiO_2-MIP



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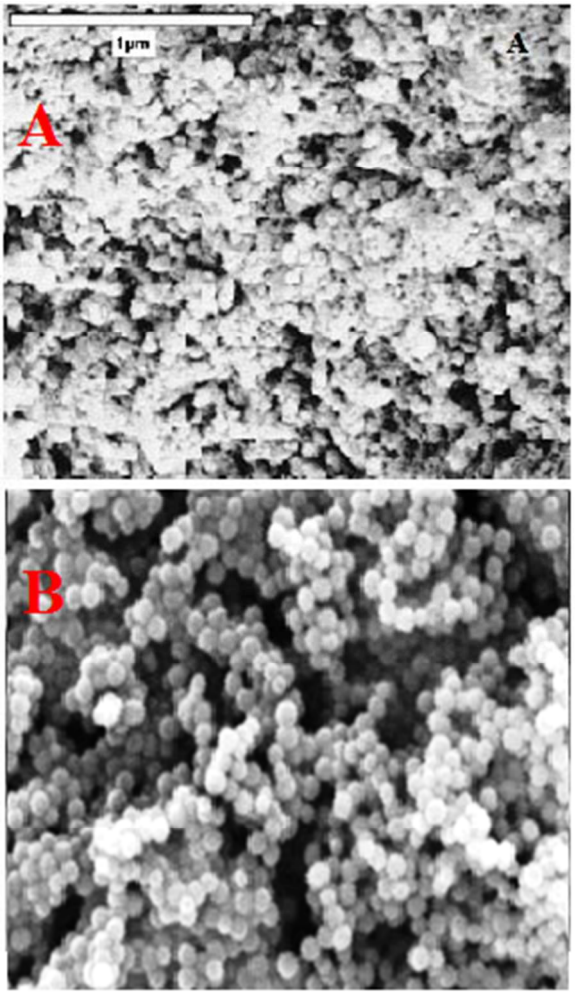

Fig. 4. SEM images of MIP (a) and SiO_2–MIP (b).

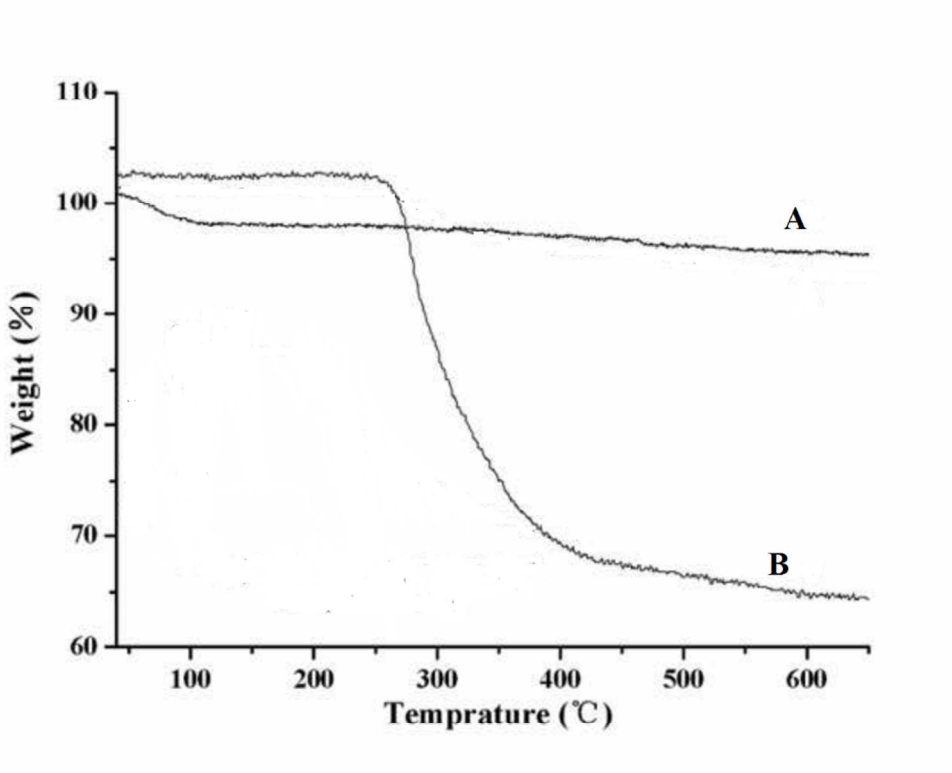

Fig. 5 shows the thermal decomposition pattern of (A) silica and (B) layer-coated silica nanoparticle

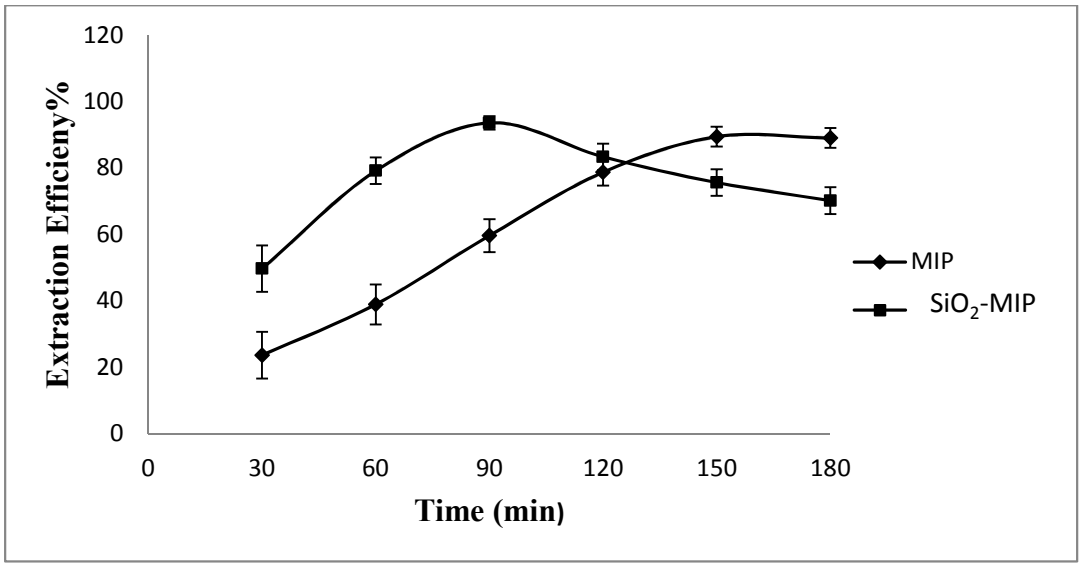

Fig. 6 Influence of adsorption time on the extraction of glycine (MIP and SiO_2–MIP)

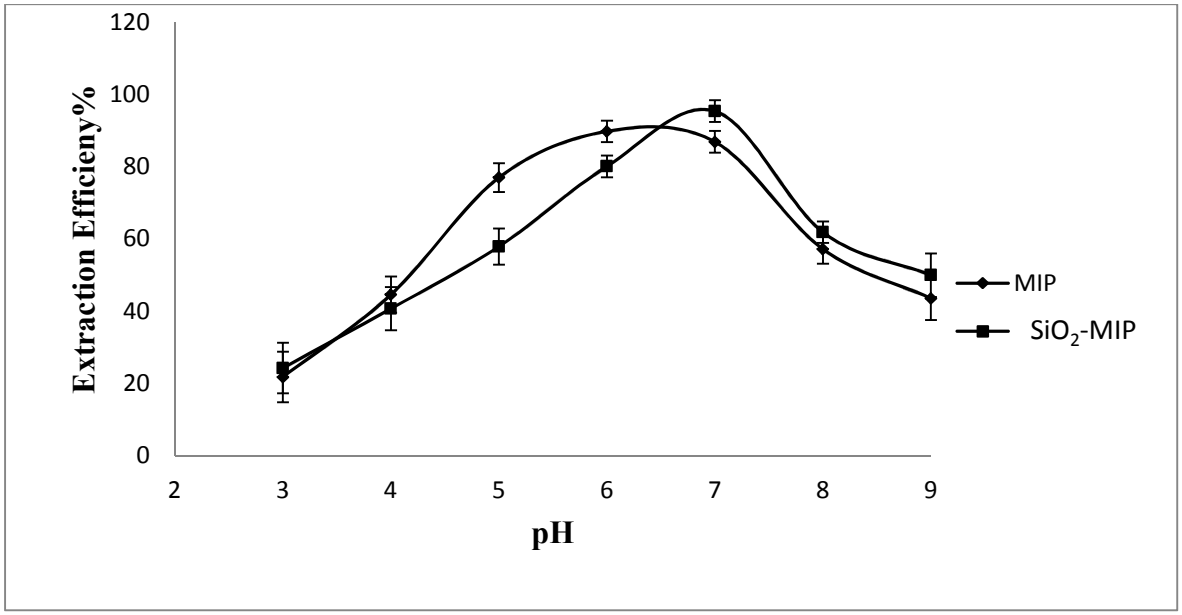

Fig. 7. Effect of pH of sample solution on glycine uptake (MIP and SiO_2–MIP)

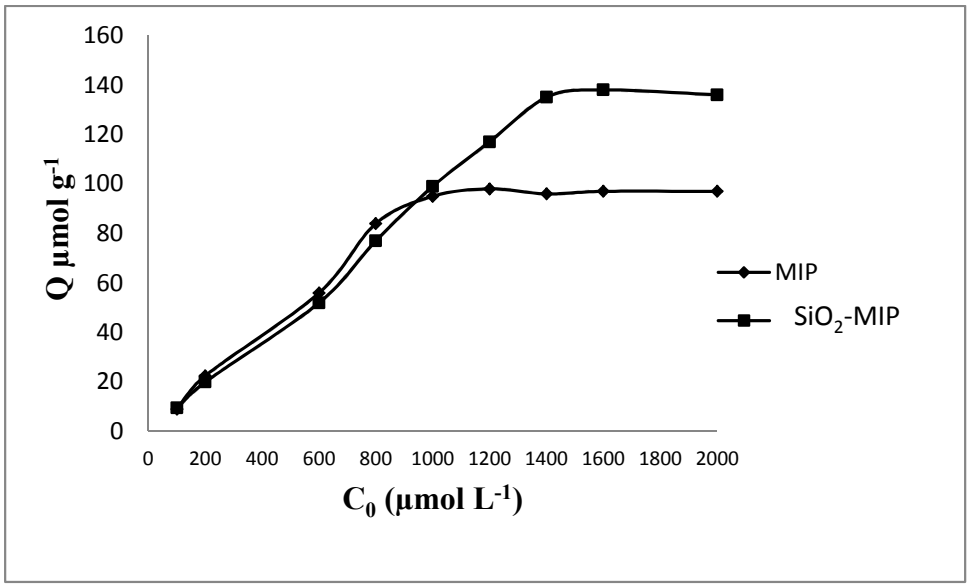

Fig. 8. The effect of glycine initial concentration on the adsorption quantity of MIP and SiO_2-MIP. Other conditions: 0.2 g of synthesized polymer, pH 7.0, shaking time 150 and 90 min for MIP and SiO_2- MIP, respectively. temperature 25 °C.

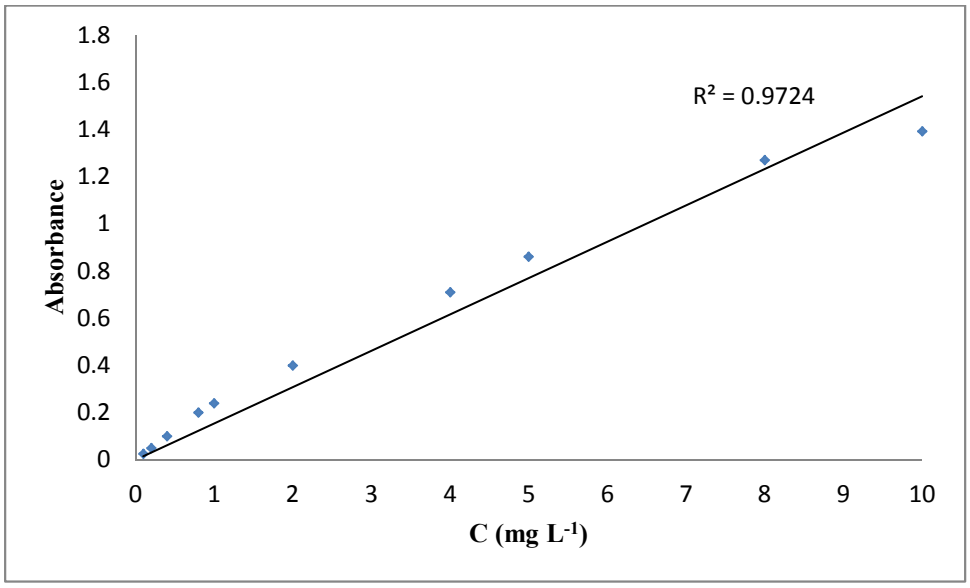

Fig. 9. Calibration curve of glycine

Table 1. Comparison of MIP, NIP, SiO_2–MIP, and SiO_2–NIP.

Polymer Type	Initial concentration/ μmol L^{-1}	Final concentration/ μmol L^{-1}	K_d	Extraction %
MIP	1000	85.1 ±1.4	1.34	91.5
NIP	1000	960.4 ±2.7	0.005	4.0
SiO_2-MIP	1000	85.2±0.8	3.44	96.5
SiO_2-NIP	1000	960.7±2.2	0.0056	4.3

Table 2. Effect of eluent type on extraction efficiency

Eluent	Recovery%
Ethanol	60.67
Water/ethanol (5/5, v/v)	93.20
Water/ethanol (7/3, v/v)	81.32
2 M acetic acid	53.46
Acetonitrile	23.59
Acetonitrile/acetic acid	43.81

Table 3. Recovery yields in the elution solution after the extraction of amino acids using MIP and SiO_2–MIP cartridges.

Amino Acid	Recovery	
	MIP-SPE	SiO_2-MIP – SPE
Glycine	87.2±0.9	93.7±0.8
Sarcosine	27.6±1.1	22.6±0.9
Alanine	15.8±0.8	11.8±0.7
Valine	6.8±0.7	5.8±0.5
lysine	8.4±1.2	6.4±0.8

Table 4. Recovery yields in the elution solution after the extraction of the binary mixture using the MIP cartridge.

Amino Acid	Glycine Recovery	
	MIP	SiO_2-MIP
Sarcosine-Glycine	74.5 ±1.5	81.4 ±1.3
Alanine- Glycine	77.2 ± 1.3	86.3 ± 1.5
Valine- Glycine	85.4 ± 1.6	90.4 ± 1.7
Lysine- Glycine	83.1 ± 1.9	91.2 ± 1.5

Table 5. Determination of glycine in human urine

	Spiked mg L^{-1}	Measured mg L^{-1}		Recovery %	
		MIP	SiO_2-MIP	MIP	SiO_2-MIP
Human urine	0	-	-	-	-
	0.5	0.42 ±0.08	0.44 ±0.09	84 ± 1.2	88±1.4
	1	0.81 ±0.07	0.89 ±0.16	81±1.5	89±1.1