Hindawi
Advances in Polymer Technology
Volume 2019, Article ID 9432412, 7 pages
https://doi.org/10.1155/2019/9432412

Research Article

Molecularly Imprinted Polymer-Silica Hybrid Particles for Biomimetic Recognition of Target Drugs

Sumaira Roshan,[1] Adnan Mujahid,[1] Adeel Afzal,[2] Izzut Nisar,[1] Mirza Nadeem Ahmad,[3] Tajamal Hussain,[1] and Sadia Zafar Bajwa[4]

[1]*Institute of Chemistry, University of the Punjab, Lahore 54590, Pakistan*
[2]*Department of Chemistry, College of Science, University of Hafr Al Batin, 31991 Hafr Al Batin, Saudi Arabia*
[3]*Department of Applied Chemistry, Government College University, Faisalabad 38030, Pakistan*
[4]*National Institute of Biotechnology and Genetic Engineering, Jhang Road, Faisalabad, Pakistan*

Correspondence should be addressed to Adnan Mujahid; adnanmujahid.chem@pu.edu.pk

Received 6 October 2018; Revised 13 January 2019; Accepted 26 February 2019; Published 1 April 2019

Academic Editor: Katja Loos

Biomimetic hybrid particles based on amlodipine-imprinted poly(methacrylic acid-co-ethylene glycol dimethacrylate) (MIP) are developed by free radical polymerization of the monomers and crosslinkers in the presence of silica nanoparticles. Atomic force microscopy is used to study the distribution and surface morphology of MIP-silica hybrid particles. The responsive properties are studied by exposing the synthesized MIP-silica hybrid material to standard amlodipine drug solution and consequently monitoring the decrease in drug concentration. The control material, i.e., nonimprinted polymer- (NIP-) silica hybrid particles, exhibits much lower response during the drug rebinding assay suggesting the lack of functionality due to the absence of imprinting effects. The selectivity of MIP-silica hybrid particles is evaluated by examining the aspirin uptake that shows lower absorbance shifts for aspirin solution compared to amlodipine. It indicates a higher sensitivity of MIP-silica hybrid particles toward targeted pharmaceutical drug recognition and also exhibits their potential for drug assay in multiplex biological samples. Furthermore, MIP-silica hybrid particles used in the drug rebinding assay can be recovered and regenerated for subsequent tests without losing recognition properties.

1. Introduction

Molecularly imprinted polymers (MIPs) [1, 2] are regarded as specific synthetic receptors as they offer structurally adapted recognition sites for rebinding of target molecules such as drugs in complex mixtures. The recognition mechanism of such molecularly imprinted materials is similar to antibody-antigen interactions, and for this reason, MIPs are often considered as the biomimetic receptors. Their simple and straightforward synthesis, structurally tailored recognition features, enhanced thermal/chemical stability, and long shelf-life make them suitable for different applications [3–5]. Some of these applications include selective extraction of target analytes from complex mixtures, biomimetic sensor coatings, enantiomeric separations, catalysis, and drug delivery. Recently [6, 7], molecular imprinted hybrid polymeric materials are developed for selective recognition of target analytes in complex samples. The conventional organic polymers prepared by bulk imprinting methods yield small amount of recoverable polymer mass after crushing/grinding having fewer recognition sites. Furthermore, these polymeric particles may swell or shrink in organic solvents leading to deformation in the shape of imprinted sites. These issues can be addressed by introducing hybrid imprinted materials having improved recognition sites with appreciable chemical/solvent resistance. For instance, Clausen et al. [8] reported the synthesis of poly(methacrylic acid)/silica hybrid polymeric particles for cholesterol recognition. In this approach, the prepolymer mixture containing methacrylic acid, methacryloxypropyltrimethoxysilane, and template molecules were combined with tetraethyl orthosilicate and followed the polymerization reaction. The coupling agent here combines the inorganic and organic phases through covalent linkage. However, in the

present strategy, we synthesized MIP-silica hybrid material by dispersing the as-prepared silica particles in prepolymer mixture of methacrylic acid, ethylene glycol dimethacrylate, and amlodipine as template and followed free radical initiated polymerization. Amlodipine besylate is taken here as target drug analyte because of its high significance in hypertensive treatment and vasodilating effect.

Calcium channel blockers such as 1,4-dihydropyridine derivatives are well known biologically active compounds, which are used for treating high blood pressure, certain types of angina pains, hypertension [9], and associated coronary heart diseases [10]. Amlodipine besylate is a relatively newer drug molecule that belongs to calcium channel blocking agents, i.e., calcium antagonist drugs, which is frequently used by patients suffering from hypertensive and coronary artery diseases both in solitary and combined dosage formulations. The increasing use of amlodipine along with other drugs also demands its selective extraction from complex mixtures and its rapid determination with sufficient accuracy. Current analytical tools including liquid and gas chromatographic techniques [11, 12], electrophoresis, enzyme-based immune assays [13], and other spectroscopic and electrochemical methods [14] offer suitable detection protocols for amlodipine determination in different matrices [15–17]. However, the extraction of target drug from complex biological samples is a challenging task for which highly selective recognition materials are needed.

In this study, MIP-silica hybrid particles are developed for biomimetic molecular recognition of amlodipine besylate. As-prepared silica particles are dispersed in the solution of functional monomers and the template followed by free-radical polymerization that yields MIP-silica hybrid particles. The morphology of developed product is characterized with atomic force microscopic images. Their recognition properties are evaluated by exposing the hybrid particles to a standard amlodipine solution and subsequently measuring the decrease in drug concentration. Moreover, their selective rebinding properties and reusability after recovery and regeneration are also investigated.

2. Experimental

2.1. Materials. All chemicals and regents were used as received from supplier without further purification. Tetraethyl orthosilicate, methacrylic acid, ethylene glycol dimethacrylate, and azobisisobutyronitrile were obtained from Sigma Aldrich, while amlodipine besylate having purity 99% is provided by Highnoon Laboratories Limited.

2.2. Synthesis of Silica Nanoparticles. Silica nanoparticles were prepared by Stober's method [18]. Briefly, 2 mL of tetraethyl orthosilicate was dissolved in 6 mL of ethanol and stirred on a hot plate at 50°C for 30 minutes. After that, liquid ammonia was added dropwise, which results in the formation of white silica precipitates. The resultant suspension was centrifuged at 4000 rpm for 10 minutes to separate silica nanoparticles. The nanoparticles were washed thoroughly with distilled water until the excessive ammonia was removed and were dried in oven for further use.

2.3. Synthesis of MIP-Silica Hybrid Particles. To prepare amlodipine-imprinted poly(methacrylic acid-co-ethylene glycol dimethacrylate-) (MIP-) silica hybrid particles, 52 μL of methacrylic acid (the monomer), 200 μL of ethylene glycol dimethacrylate (the crosslinker), and 10 mg of amlodipine (the template) were mixed together in methanol along with the calculated amount of silica nanoparticles. The mixture was stirred for 30 minutes at 60°C and then 15 mg of azobisisobutyronitrile (the initiator) was added to start the free radical polymerization reaction. After two hours of continuous magnetic stirring and heating, the MIP-silica hybrid particles were removed by centrifugation. A schematic representation of the formation of MIP-silica hybrid particles is shown in Figure 1. The nonimprinted (NIP-silica hybrid) particles are also prepared as control or reference material using a similar method but without the addition of template molecules.

2.4. Characterization and Molecular Recognition Properties of MIP-Silica Hybrid Particles. The surface morphology of MIP-silica hybrid particles was characterized by SHIMADZU WET-SPM 9600 atomic force microscope. The molecular recognition properties of MIP-silica hybrid particles were studied by exposing them to standard amlodipine solution and measuring the absorbance of this solution before and after amlodipine uptake, which gives the direct information about rebinding of amlodipine. For rebinding experiments, 15 mg of thoroughly washed and dried amlodipine-imprinted-silica hybrid particles was treated against 10 mL standard amlodipine solution and the mixture was stirred for 15 minutes. Afterwards, MIP-silica hybrid particles were separated by centrifugation and the supernatant was analyzed by Labomed UVD 3500 spectrometer. The rebinding tests were conducted in the concentration range of 10-80 ppm. All the standard amlodipine solutions were prepared in methanol and their absorbance before and after amlodipine uptake was monitored at 360 nm. The rebinding tests were carried out at room temperature. The same practice was carried out for NIP-silica hybrid particles to quantify the nonspecific interactions.

The selectivity and cross-sensitivity of MIP-silica hybrid particles were observed by exposing them to aspirin, i.e., an interfering/competing drug often used in combination with amlodipine. For selectivity test, 15 mg of amlodipine-imprinted-silica hybrid particles was treated against 10 mL standard aspirin solution, i.e., 30 ppm at room temperature. The mixture was stirred for 15 minutes and then particles were separated by centrifugation. The supernatant was analyzed at 230 nm by Labomed UVD 3500 spectrometer. The absorbance of standard aspirin solution before and after treating with amlodipine-imprinted-silica hybrid particles was monitored. The relative absorbance shift for aspirin uptake and rebinding was compared with that of amlodipine to show the selective nature of MIP-silica hybrid particles.

Furthermore, these MIP-silica hybrid particles, used in the first phase of drug rebinding studies, were regenerated by thoroughly washing with distilled water and methanol. This procedure helps recovering the imprinted cavities within

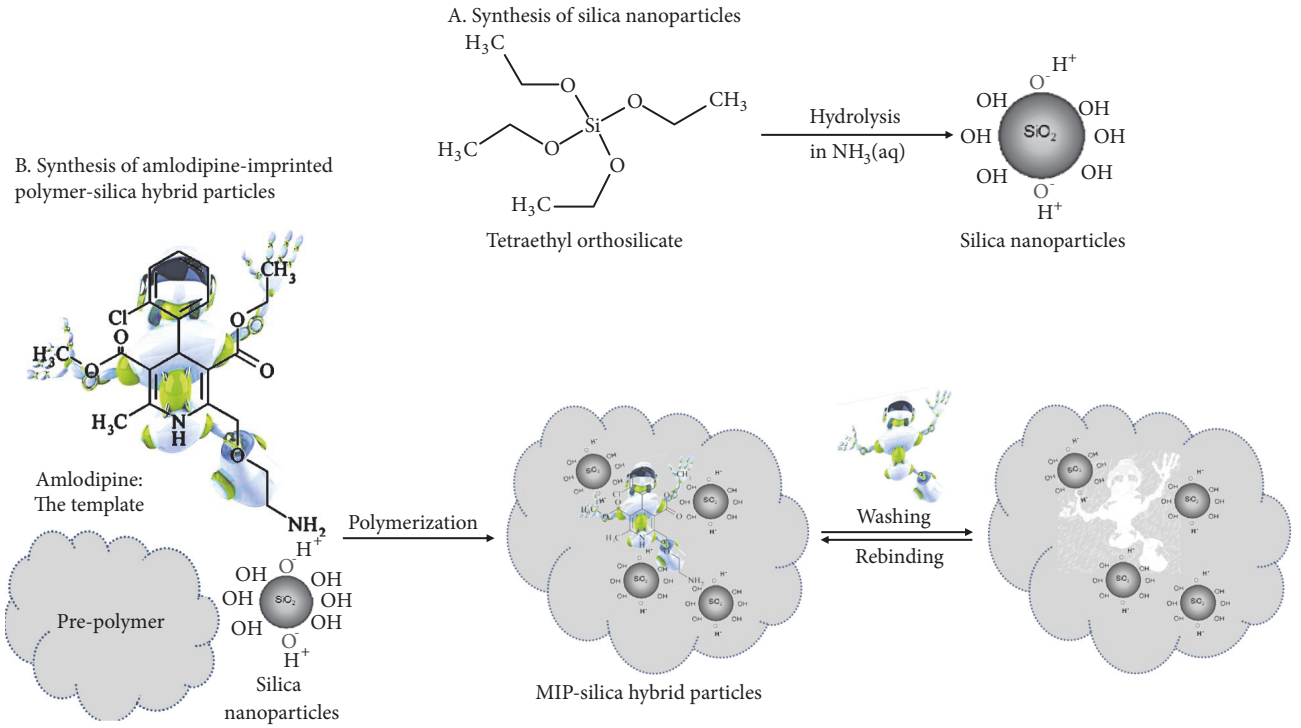


Figure 1: A schematic representation of the formation of MIP-silica hybrid particles.

the MIP-silica hybrid particles by removing adsorbed drug molecules. The thoroughly washed MIP-silica hybrid particles were collected by centrifuging the mixture at 4000 rpm. Subsequently, the regenerated particles were dried and tested again for the next round of rebinding experiments where the regenerated MIP-silica hybrid particles were treated against 15 mL standard amlodipine solution, i.e., 60 ppm at room temperature. The absorbance of standard amlodipine solution after treating with regenerated MIP-silica hybrid particles was recorded. The resultant absorbance shifts for regenerated and freshly prepared MIP-silica hybrid particles were compared to calculate the percentage response of regenerated particles.

3. Results and Discussion

The as-prepared silica nanoparticles and amlodipine-imprinted MIP-silica hybrids are coated on quartz surface as thin films to study the surface morphology and particles distribution. The respective atomic force micrographs are shown in Figure 2. AFM image and 3-D surface map of silica nanoparticles exhibit uniform size distribution and surface roughness with a maximum height (Z-scale) of 12 nm. The size of as-prepared silica particles is small (39 ± 14 nm). On the other hand, MIP-silica hybrid particles prepared via free-radical polymerization of the monomers in the presence of template and silica nanoparticles have higher surface roughness and relatively bigger size (248 ± 74 nm). This is understandable due to the tendency of silica particles to agglomerate in the prepolymer matrix and the formation of polymer-silica hybrids.

Nonetheless, AFM image and 3D surface map of MIP-silica hybrids also demonstrate uniform surface morphology with a maximum height (Z-scale) of 80 nm. These MIP-silica hybrid particles contain highly adapted interaction sites for target drug's rebinding thus, leading to improved recognition. Furthermore, the integration of silica nanoparticles with the imprinted polymer increases the number of reachable interaction centers comparing to bulk imprinted particles. This reduces the length of diffusion pathways, which favors faster mass transfer rates during the drug uptake as well as complete recovery and regeneration of the occupied cavities after washing out the analyte.

The developed MIP-silica hybrid particles were subjected to successive washings with methanol to remove the template: amlodipine. The supernatant of each washing was analyzed by UV/Vis spectroscopy which showed a decrease in absorbance because of the washed-out template. It was observed that the absorbance of amlodipine approached zero or negligible after four washings, as shown in Figure 3(a). This ensured complete removal of the template, thus leaving behind structurally adapted cavities or interactions sites within the MIP-silica hybrids. Thoroughly washed and dried MIP-silica hybrid particles were subsequently tested to evaluate their amlodipine-rebinding characteristics by exposing them to the target drug in a standard solution of amlodipine. After treatment with hybrid particles, the absorbance of standard amlodipine solution decreases. This suggests that amlodipine molecules are taken up and adsorbed by the MIP-silica hybrid particles leading to a decrease in the concentration of amlodipine in standard solution. Figure 3(b) shows a plot of the similar

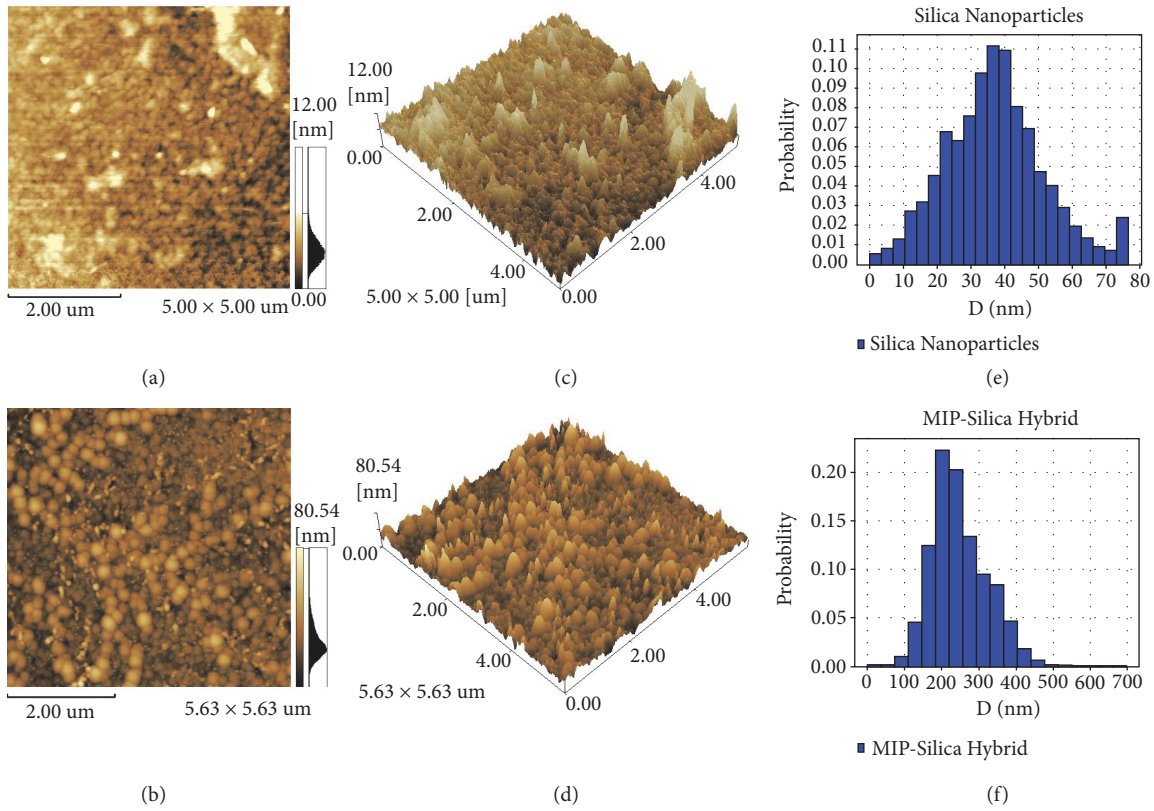


FIGURE 2: AFM micrographs of the as-prepared silica nanoparticles (a) and MIP-silica hybrid particles (b) showing nanoparticles distribution and surface morphology. The respective height scale 3-dimensional micrographs (c, d) and the histograms (e, f) of as-prepared silica nanoparticles and MIP-silica hybrid particles are also shown.

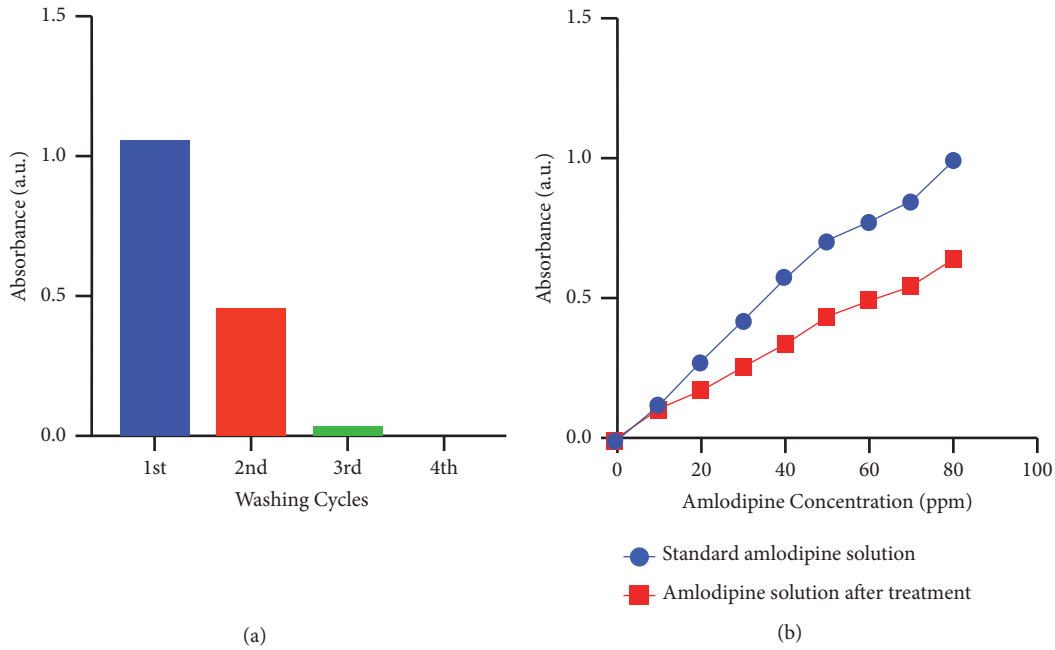


FIGURE 3: (a) The decreasing absorbance of amlodipine on successive washings confirms the removal of template molecules from the MIP-silica hybrid particles, whereas (b) shows the absorbance of standard amlodipine solutions (concentration: 10-80 ppm) decreasing after treatment with the hybrid particles.

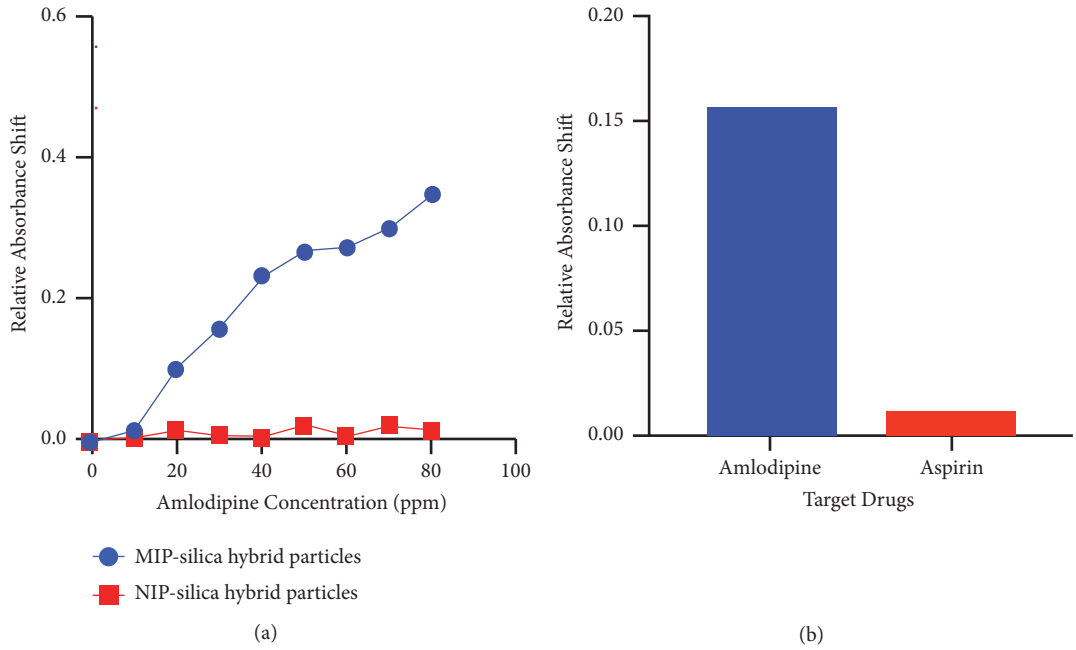


Figure 4: (a) A comparison of the relative absorbance shifts of standard amlodipine solution treated with MIP-silica and NIP-silica hybrid particles, respectively, while (b) shows the relative absorbance shifts for amlodipine and aspirin drugs after treatment with MIP-silica hybrid particles: The greater absorbance shift for amlodipine represents selectivity of the particles.

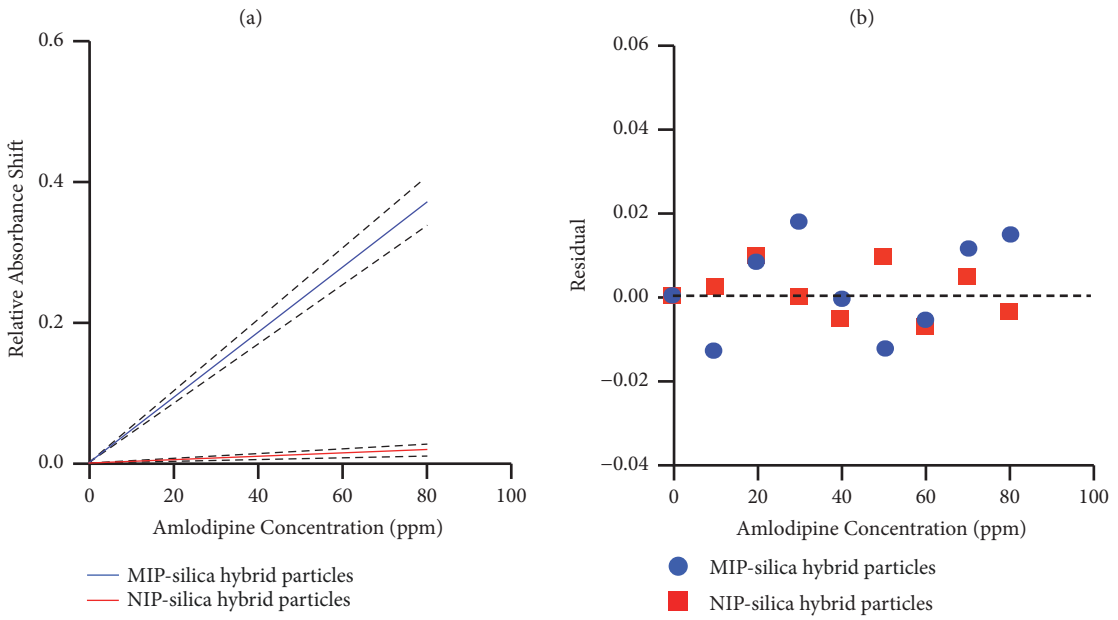
behavior observed at a range of amlodipine concentrations (10-80 ppm).

A comparison of the relative absorbance shifts of standard amlodipine solutions treated with imprinted (MIP-silica) and nonimprinted (NIP-silica) hybrid particles is provided in Figure 4(a). MIP-silica hybrid particles possess structurally adapted sites for amlodipine recognition, which leads to greater drug uptake and a noticeable decrease in the absorbance of standard solution. Instead, NIP-silica hybrid particles (prepared without addition of the template) lack such functionality or sterically adapted interaction sites for amlodipine, which means they can act as control material to compare the nonspecific binding interactions. That is why the absorbance shift for MIP-silica hybrid particles was much higher compared to NIP-silica hybrid material.

The selectivity of MIP-silica hybrid particles was evaluated by observing their cross-sensitivity toward another drug, for example, aspirin. The relative shifts in the absorbance of standard solutions of aspirin and amlodipine were recorded and compared, as shown in Figure 4(b). It was observed that shift in the absorbance of amlodipine solution was 10 times higher than aspirin solution, which indicated that MIP-silica hybrid particles were more responsive and highly selective toward target drug recognition. It is because the imprinted cavities possess sterically and chemically adapted interaction sites, which facilitates the target drug uptake and rebinding. On the other hand, aspirin is not recognized by MIP-silica hybrid particles due to its structural mismatch, i.e., different size as well as chemical functional groups, with the imprinted cavities.

The sensitivity of MIP-silica hybrid particles was quantitatively measured from the calibration curves drawn by the linear regression analysis of the absorbance data. Figure 5(a) shows the straight lines for MIP-silica and NIP-silica hybrid particles along with 95% confidence bands of the best-fit line. The residual plot in Figure 5(b) represents the notion that the linear regression model is appropriate for the data since the residual points are randomly dispersed against the horizontal line. The data obtained from the linear regression analysis is also presented. The sensitivity of both MIP-silica and NIP-silica hybrid particles was calculated from the slope of the best-fit straight lines in Figure 5(a). MIP-silica hybrid particles demonstrated excellent sensitivity (0.00467 ± 0.00017 ppm^{-1}) toward amlodipine that was 19.7 times higher than NIP-silica hybrid particles (0.00024 ± 0.00004 ppm^{-1}). In a similarly, the sensitivity of MIP-silica hybrid particles was measured toward aspirin that was found to be 0.00013 ± 0.00002 ppm^{-1}. Again, MIP-silica hybrid particles revealed 35.3 times higher sensitivity toward amlodipine (the target drug) compared to aspirin, which signifies the greater selectivity and low cross-sensitivity of the hybrid particles.

The target drug recognition by MIP-silica hybrid particles is achieved through noncovalent receptor-drug interactions, which may include hydrogen bonding, dipolar interactions, or others. Therefore, these receptors can be reused several times for rebinding tests. The reusability of the developed product was evaluated by recovering the particles that have already been used for drug rebinding tests and regenerating the imprinted cavities by thorough washing. The MIP-silica hybrid particles were washed with methanol several times to remove already captured drug molecules. Subsequently, these regenerated particles were exposed to 60 ppm standard amlodipine solution again and, subsequently, the decrease in



Results	MIP-silica hybrid particles	NIP-silica hybrid particles
Slope	0.00467	0.00024
Standard error	0.00017	0.00004
95% confidence interval's slope	0.00427 to 0.00506	0.00014 to 0.00034

FIGURE 5: (a) A plot showing linear regression analysis of the absorbance data of MIP-silica hybrid and NIP-silica hybrid particles along with 95% confidence bands. (b) The residual plot showing the residuals distribution along the horizontal axis.

absorbance was monitored. The relative absorbance shifts of regenerated and freshly prepared MIP-silica hybrid particles were compared. The regenerated MIP-silica hybrid particles demonstrated a high rebinding efficiency; i.e., they retain around 95% of the sensitivity toward amlodipine compared to freshly prepared particles. The results suggest that the developed MIP-silica hybrid particles can be effectively used for successive rounds of drug uptake and rebinding tests without losing the essential molecular recognition properties.

4. Conclusion

Molecular imprinting is an efficient method for producing biomimetic receptor materials with tuned functionality and molecular recognition properties. Their synthetic procedure is uncomplicated, and they can be tailored for a diverse range of analytes. They possess selective binding sites, which suggest that these materials can be used for target drug assays in complex biological matrices. This property makes MIPs low-cost potential receptors viable for target drug recognition. Furthermore, they can be regenerated and reused for successive rebinding experiments without losing recognition properties. MIP-silica hybrid particles are easy to synthesize and show good sensitivity, selectivity, and reusability and, thus, can be used for amlodipine drug recognition in complex fluids.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank Highnoon Laboratories for providing amlodipine besylate and aspirin; furthermore, we are also

thankful to University of the Punjab, Pakistan, for the research funds to carry out this work.

References

[1] V. George, L. I. Andersson, R. Müller, and K. Mosbach, "Drug assay using antibody mimics made by molecular imprinting," *Nature*, vol. 361, no. 6413, pp. 645–647, 1993.

[2] A. Cameron, H. S. Andersson, L. I. Andersson et al., "Molecular imprinting science and technology: a survey of the literature for the years up to and including," *of Molecular Recognition: An Interdisciplinary Journal*, vol. 19, no. 2, pp. 106–180, 2006.

[3] W. Jinfang, P. A. G. Cormack, D. C. Sherrington, and E. Khoshdel, "Monodisperse, molecularly imprinted polymer microspheres prepared by precipitation polymerization for affinity separation applications," *Angewandte Chemie*, vol. 115, no. 43, pp. 5494–5496, 2003.

[4] W. Judith and R. Schirhagl, "Applications of Molecularly imprinted polymer nanoparticles and their advances toward industrial use: a review," *Analytical Chemistry*, vol. 88, no. 1, pp. 250–261, 2015.

[5] C. Lingxin, X. Wang, W. Lu, X. Wu, and J. Li, "Molecular imprinting: perspectives and applications," *Chemical Society Reviews*, vol. 45, no. 8, pp. 2137–2211, 2016.

[6] L. v. Yun-Kai, L.-M. Wang, S.-L. Yan, X.-H. Wang, and H.-W. Sun, "Synthesis and characterization of molecularly imprinted poly(methacrylic acid)/silica hybrid composite materials for selective recognition of lincomycin in aqueous media," *Journal of Applied Polymer Science*, vol. 126, no. 5, pp. 1631–1636, 2012.

[7] L. v. Yun-Kai, L.-M. Wang, L. Yang, C.-X. Zhao, and H.-W. Sun, "Synthesis and application of molecularly imprinted poly(methacrylic acid)-silica hybrid composite material for selective solid-phase extraction and high-performance liquid chromatography determination of oxytetracycline residues in milk," *Journal of Chromatography A*, vol. 1227, pp. 48–53, 2012.

[8] C. D. Nobile, I. M. R. Pires, and C. R. T. Tarley, "Improved selective cholesterol adsorption by molecularly imprinted poly(methacrylic acid)/silica (PMAA-SiO2) hybrid material synthesized with different molar ratios," *Materials Science and Engineering: C*, vol. 44, pp. 99–108, 2014.

[9] Y. José-Miguel, S. Oparil, B. R. Davis et al. et al., "Stroke outcomes among participants randomized to chlorthalidone, amlodipine or lisinopril in ALLHAT," *of the American Society of Hypertension*, vol. 8, no. 11, pp. 808–819, 2014.

[10] S. De Portu, E. Menditto, L. Scalone, S. Bustacchini, C. Cricelli, and L. G. Mantovani, "The pharmacoeconomic impact of amlodipine use on coronary artery disease," *Pharmacological Research*, vol. 54, no. 2, pp. 158–163, 2006.

[11] A. B. Baranda, C. A. Mueller, R. M. Alonso, R. M. Jiménez, and W. Weinmann, "Quantitative determination of the calcium channel antagonists amlodipine, lercanidipine, nitrendipine, felodipine, and lacidipine in human plasma using liquid chromatography-tandem mass spectrometry," *Therapeutic Drug Monitoring*, vol. 27, no. 1, pp. 44–52, 2005.

[12] J. Bhatt, S. Singh, G. Subbaiah, B. Shah, S. Kambli, and S. Ameta, "A rapid and sensitive liquid chromatography-tandem mass spectrometry (LS-MS/MS) method for the estimation of amlodipine in human plasma," *Biomedical Chromatography*, vol. 21, no. 2, pp. 169–175, 2007.

[13] K. Matalka, T. El-Thaher, M. Saleem, T. Arafat, A. Jehanli, and A. Badwan, "Enzyme linked immunosorbent assay for determination of amlodipine in plasma," *Journal of Clinical Laboratory Analysis*, vol. 15, no. 1, pp. 47–53, 2001.

[14] C. F. Valezi, E. H. Duarte, G. R. Mansano, L. H. Dall'Antonia, C. R. T. Tarley, and E. R. Sartori, "An improved method for simultaneous square-wave voltammetric determination of amlodipine and enalapril at multi-walled carbon nanotubes paste electrode based on effect of cationic surfactant," *Sensors and Actuators B: Chemical*, vol. 205, pp. 234–243, 2014.

[15] R. N. Goyal and S. Bishnoi, "Voltammetric determination of amlodipine besylate in human urine and pharmaceuticals," *Bioelectrochemistry*, vol. 79, no. 2, pp. 234–240, 2010.

[16] M. Amiri and H. Imanzade, "Adsorption of amlodipine at the surface of tosyl—carbon nanoparticles for electrochemical sensing," *Iranian Journal of Pharmaceutical Research*, vol. 15, no. 3, pp. 303–311, 2016.

[17] H. Beitollahi, F. Ebadinejad, F. Shojaie, and M. Torkzadeh-Mahani, "A magnetic core–shell Fe 3 O 4@ SiO 2/MWCNT nanocomposite modified carbon paste electrode for amplified electrochemical sensing of amlodipine and hydrochlorothiazide," *Analytical Methods*, vol. 8, no. 32, pp. 6185–6193, 2016.

[18] W. Stöber, A. Fink, and E. Bohn, "Controlled growth of monodisperse silica spheres in the micron size range," *Journal of Colloid and Interface Science*, vol. 26, no. 1, pp. 62–69, 1968.