PAPER DETAILS

TITLE: Beta Glucosidase Recognition By Imprinted Polyacrylamide Hydrogels

AUTHORS: Mervecan SEVILDIK, Ayse DINÇER, Tülin AYDEMIR

PAGES: 17-23

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/766623



Eurasian Journal of Biological and Chemical Sciences



Beta Glucosidase Recognition By Imprinted Polyacrylamide Hydrogels

Mervecan Sevildik¹, Ayşe Dinçer ^{1*} , Tülin Aydemir ¹

¹ Manisa Celal Bayar University, Faculty of Science and Arts, Chemistry Department, Manisa, Türkiye.

*Corresponding author : ayse.dincer@cbu.edu.tr Orcid No: 0000-0001-6158-1775 Received : 04/01/2019 Accepted : 30/09/2018

Abstract: In the present work, selective adsorption of β -glucosidase using imprinted polyacrylamide hydrogels were studied. For this purpose imprinted hydrogels were prepared using β -glucosidase as a template molecule, acrylamide (AAm) as a monomer, N,N'-methylenebisacrylamide (MBAA) as a crosslinker, ammonium persulphate (APS) and N,N,N',N'-tetramethylethylene-diamine (TEMED) as initiators. β -Glucosidase imprinted hydrogel was washed with a solution of sodium dodecyl sulfate (SDS) and acetic acid to remove the template molecule. Non-imprinted hydrogel was also prepared without using β -glucosidase template. The adsorption and recognition performance of hydrogels towards β -glucosidase was discussed through adsorption isotherms and adsorption kinetics. In batch template rebinding experiments, imprinted hydrogels displayed quite high template binding capacity than non-imprinted hydrogels. The theoretical maximum adsorption capacity (Qmax) was determined by the Langmuir model, which turned out to be 7.2 mg/g and 4.6 mg/g for imprinted and non-imprinted hydrogels, respectively. A pseudo-second-order model was suitable to interpret kinetic data. Imprinted polyacrylamide hydrogels also used as an affinity sorbent for selective β -glucosidase adsorption from almonds.

Keywords: β-Glucosidase, polyacrylamide, hydrogel, molecular imprinting.

© EJBCS. All rights reserved.

1. Introduction

Molecular imprinting is regarded as a promising technique for preparing artificial receptors with "tailor-made"binding sites for specific binding the template molecular by polymerizing a mixture of appropriate functional monomer, befitting cross-linker and template. (Zhao et al. 2014, Pisarev and Polyakova 2018, Wang et al. 2018) Molecularly imprinted polymers (MIPs) are characterized by their thermal and chemical stability, high specificity, ease of mass preparation, low cost and reusability, which promote their wide applications in chromatography, catalysis, sensors, drug release and environmental protection. (Zaidi 2018, Li et al. 2005) There are several challenges remain in bio-macromolecule imprinting, such as those involving proteins, DNAs, and even whole cells and viruses. Manv inherent problems of biomacromolecules hinder the advancement of their imprinting, such as large molecular size, structural complexity, environmental sensitivity, and flexible conformation (Nishino et al. 2006, Lv et al, 2013). The use of hydrogel with soft and macroporous structure is beneficial in maintaining the native protein conformation. Imprinting within hydrogels presents the possibility of three dimensional binding sites that eventually should lead to improved selectivity, while imprinting in an aqueous phase ensures solubility and conformational stability of the template proteins (Kimhi and Bianco-Peled, 2007).

Polyacrylamide (PAM) hydrogel was widely used as the matrix for the molecular imprinting of proteins. PAM hydrogel is biocompatible and has soft and wet macroporous structure. The abundant amide functional groups in PAM can form strong interactions with peptide bonds in the proteins even in aqueous system (Zhao et al. 2015). β -Glucosidase (EC 3.2.1.21) is a type of hydrolase widely existing in various sources such as microorganisms, plant and animal tissues. It catalyzes the hydrolysis of βglycosidic linkages in di- and oligoglucosaccharides and several other glycol conjugates (Verma et al. 2011, Çelik et al. 2016). In this study, imprinted polyacrylamide hydrogels for recognition of β -glucosidase were prepared and tested for β -glucosidase rebinding. Effect of contact time, pH, temperature, initial β -glucosidase concentration on rebinding of β -glucosidase on MIP and NIP hydrogels were investigated. To investigate the nature of the adsorption process, the non-linear Langmuir and Freundlich isotherms were used to fit the equilibrium adsorption data. The kinetic data have been analyzed using a pseudofirst-order, pseudo-second-order equations. β-glucosidase imprinted polyacrylamide hydrogels also used as an affinity matrix for selective adsorption of the enzyme from almonds. Purity controls were done using SDS-PAGE.

2. MATERIALS AND METHOD

2.1 Materials

 β -glucosidase from almonds (≥ 2 U/mg solid), acrylamide (AAm), N,N'-methylene bisacrylamide (MBA), ammonium persulfate and N,N,N',N'-tetramethylethylene-diamine (TEMED) were supplied from Sigma-Aldrich.

All other chemicals used in this study were in analytical grade.

2.2 Method

2.2.1 Preparation of β -glucosidase imprinted hydrogel

Molecularly imprinted hydrogels (MIP) were synthesized by dissolving AAm (54 mg), and MBA as a crosslinker (6 mg), respectively along with template protein β -glucosidase (10 mg) in 1 mL of phosphate buffer (0.1 M, pH 7.0). The prepolymerization solution was purged with nitrogen gas for 10 min. to remove dissolved oxygen followed by an addition of 10 mg APS and 20 µL of a 5% (v/v) TEMED solution. Polymerization occurred overnight at room temperature. The template molecule β -glucosidase was eluted from hydrogel with acetic acid solution (10%, v/v) containing SDS (10%, w/v) until no protein in the supernatant was detected. Protein measurements were done by Bradford method. Ultimately, the hydrogel was extensively washed with deionized water to remove remnant SDS and acetic acid. Non-imprinted hydrogel (NIP) was also prepared, which corresponds to imprinted hydrogel (MIP) but without the template. All the MIP and NIP hydrogels were dried at 50 °C for 12 h before use.

2.2.2 Adsorption studies

β-glucosidase rebinding onto imprinted and non-imprinted hydrogels were carried out using a batchwise adsorption method. In each rebinding experiments, enzyme solution (1 mL) prepared in buffer and 0.01 g of dried imprinted and nonimprinted hydrogels were used. The experiments were conducted to observe the effect of various parameters such as contact time, temperature, pH and enzyme concentration. The effect of contact time on β -glucosidase adsorption was studied by varying the contact time from 15 to 120 min. The effect of the temperature on the adsorption was studied at various temperature values (25-40°C) accompanied by mild shaking. Effect of enzyme concentration on the adsorption was also recorded in the concentration range from 1-3.5 mg/mL. After adsorption, the hydrogels were removed by centrifugation and the supernatant was analyzed for the unadsorbed enzyme spectrophotometrically.

The amount of enzyme adsorbed on the hydrogels were calculated according to the following equation 1:

$$qe = \frac{(C_0.V_0 - Ce.V)}{m}$$
(1)

where qe is the amount of protein adsorbed onto unit mass of dry gel (mg/g), C_0 and Ce are the initial and equilibrium concentrations of protein (mg/mL), respectively, V_0 and V is

volume (mL) of the initial and final enzyme solution containing the hydrogel and m is the amount of dry hydrogel (g) used in the experiment (Bhattacharyya and Ray 2013).

The specific recognition property of the imprinted hydrogel is illustrated by the imprinting factor (IF) according to the Kempe and Mosbach 1994, which is defined as:

$$IF = \frac{q_{imprinted}}{q_{nonimprinted}} \quad (2)$$

where $q_{imprinted}$ is the amount of protein on the imprinted hydrogel (mg/g), and $q_{non-imprinted}$ is the amount of protein on the non-imprinted hydrogel (mg/g).

2.2.3 Partial purification of β -glucosidase from almonds

Partial purification of β -glucosidase from almonds was done according to the method of Ergöçen (2013). In brief, 8 mL of chilled acetone (-20 °C) was added to each gram of almonds extracted from hard shells and homogenized in porcelain mortar. By filtering the homogenate through filter paper, the phenolic compounds and some lipids were removed together with the filtrate. The remaining portion of the sample on filter paper was taken back to the porcelain mortar and the process was repeated 3 times as described above. As a result of the final filtration, the remaining solid in the filter paper was stored at 5 °C for 24 hours to evaporate acetone. 1 g of the solid extract obtained after acetone extraction was dissolved in 50 mL of citric acid (50 mM, pH 5) buffer and 60% (NH₄)₂SO₄ precipitation was applied. The precipitate obtained after the ammonium sulfate precipitation was suspended in 50 mM pH 5.0 citrate buffer (4 mL) and dialyzed. The dialyzed enzyme solution applied on MIP and NIP hydrogels and incubated for 120 min. Recovery of the β -glucosidase from the hydrogels were performed using 50 mM pH 5.0 citrate buffer. βglucosidase activities and protein contents were measured in every step and applied to SDS-PAGE.

3. Results and Discussions

3.1 β-glucosidase imprinted hydrogel

The polyacrylamide matrix is non-charged and multiple weak interactions, like hydrogen bonds and dipole-dipole interactions are assumed to be responsible for the polymertemplate interactions (Tong et al. 2001). Proteins are very complex and possess many potential recognition sites at their surface, such as charged amino acids and hydrophobic/hydrophilic regions (Verheyen et al. 2011). The polymerization of monomers in the vicinity of a template protein leads to the formation of a cavity with the shape and size of the imprinted template, and with the sites of interaction in a pre-determined orientation (Hjerten et al. 1997, Ghasemzadeh et al. 2008). It is well known that the diffusivity of proteins in a highly crosslinked polymer matrix is rather slow. Polymer geometry, polymer hydration, crosslink density, protein size and temperature all play a role in the time needed for a protein to diffuse into the polymer matrix and to reach equilibrium (Verheyen et al. 2011).

β-Glucosidase from almonds is a homodimer consisting of 2 equal subunits of 65 kDa and is a glycoprotein. It has an isoelectric value of 7.3 and 6.7 nm molecular diameter (Grover et al. 1977, Gómez et al. 2012). Hydrogen bonding is the dominant driving force for the molecular recognition between β-glucosidase and the monomers (Fig. 1). Multiple-point hydrogen bonds could be produced when the cavities in the hydrogel had complementary interaction surface with the protein (Pan et al. 2009). Elution of β-glucosidase from polyacrylamide gel was performed using (pH 2.8). Negatively charged SDS micelles were interacted positively charged protein at this pH. Acetic acid could disrupt intramolecular hydrogen bonds within the protein (Hawkings et al. 2005)



Figure 1. Proposed mechanism of a) NIP hydrogel b) β -glucosidase imprinted hydrogel (Adapted from Pan et al. 2009)

3.2 Characterization of the Imprinted and Non-imprinted Hydrogels

3.2.1 SEM Analysis

Scanning electron microscopy (SEM) has been an essential tool for characterizing the surface morphology and fundamental physical properties of the adsorbent. It is useful for determining the particle shape, porosity and appropriate size distribution of the adsorbent. As seen from Figure 2, unwashed MIP and NIP hydrogel have smooth and porous surface. To remove the template protein, MIP hydrogel was washed with SDS:AcOH 10% (w/v):10% (v/v) and then the surface of MIP hydrogel became rough and more porous.



Figure 2. SEM micrographs of a) Unwashed MIP hydrogel (30000x) b) Washed MIP hydrogel (30000x) c) NIP hydrogel (10000x)

3.3 Optimization of Rebinding Conditions

3.2.1 Effect of contact time

The contact time between the enzyme and the hydrogel is an important parameter in enzyme separation by adsorption. 2.5 mg/mL β-glucosidase in phosphate buffer (0.1 M pH 7.0) was added to 0.01 g of hydrogel and incubated for determined time intervals. Fig. 3 shows the relationship between the adsorption capacity of MIP hydrogel (NIP) and the adsorption time. At room temperature, within the first 15 min the adsorption capacity increases rapidly. The rebinding reaches a plateau after about 120 min, indicating that the imprinted sites are saturated with β -glucosidase. The adsorption capacity of NIP hydrogel is much smaller than that of the MIP hydrogel. MIP hydrogels have adsorption cavities which are complementary to the template molecule in shape. Comparing this MIP with the NIP hydrogel, it is clear that a much higher rebinding capacity is achieved.



Figure 3. Effect of contact time on rebinding of β -glucosidase on MIP and NIP hydrogels.

3.3.2 Effect of pH

In this study, series of 2.5 mg/mL β -glucosidase solution were prepared in different pH values (0.1 M pH 3.0-4.0 acetate buffer, 0.1 M pH 5.0-6.0 citrate buffer, 0.1 M pH 7.0 phosphate buffer, 0.1 M pH 8.0 Tris-HCl buffer) and added on the hydrogels. After incubation at 25°C for 120 minutes, the amount of protein adsorbed on MIP and NIP hydrogels were calculated. As seen from Figure 4, the imprinting efficiency reached the maximum when the pH was 7.0 because the pH value affected the β -glucosidase conformation and charge.



Figure 4. Effect of pH on rebinding of β -glucosidase on MIP and NIP hydrogels (contact time: 120 min, enzyme concentration 2.5 mg/mL).

3.3.3 Effect of temperature

In this study 2.5 mg/mL β -glucosidase solution (0.1 M pH 7.0 1 mL) was added to the hydrogels and shaken gently at various temperature values (25-40 °C) for 120 minutes. As seen from Figure 5, the adsorption of the enzyme β -glucosidase decreased with the increase in temperature. At 25°C it appears that MIP hydrogels have higher β -glucosidase selectivity than NIP hydrogels. β -glucosidase adsorption decreased with increasing temperature suggesting that the interactions between β -glucosidase and the hydrogel were based on hydrogen bonds.



Figure 5. Effect of temperature on rebinding of β -glucosidase on MIP and NIP hydrogels (contact time: 120 min, enzyme concentration 2.5 mg/mL, pH 7.0).

3.3.4 Effect of initial β -glucosidase enzyme concentration

The adsorption isotherm experiments for MIP and NIP were carried out using different β -glucosidase concentrations in the range 1-3.5 mg/mL for 120 min accompanied by mild shaking. The other operational parameters (contact time, pH and temperature) were kept at optimum values. At low different β -glucosidase concentrations, was not enough to fill up the specific binding cavities (Fig. 6). The specific imprinted sites were gradually occupied and the adsorption capacity of MIP hydrogel became steady with increasing different β -glucosidase concentration, and the saturation value was achieved at β-glucosidase concentration of 3.5 mg/mL. The maximum adsorption capacities of MIP and NIP hydrogel were 3.74 and 2.53 mg/g, respectively and the IF value was calculated as 1.47. The results showed that MIP hydrogel had a higher adsorption capacity for βglucosidase than that of NIP hydrogel.



Figure 6. Effect of initial enzyme concentration on rebinding β -glucosidase on MIP and NIP hydrogels. (contact time: 120 min, temperature: 25 °C, pH 7.0).

3.4 Adsorption Isotherms

Equilibrium study is important as it provides the qualitative information on the nature of solute-solid surface interactions and could be used to evaluate the adsorption capacity of a particular adsorbent. In this study, two widely used isotherm models, i.e., Langmuir model and Freundlich model were used to describe the adsorption process. These isotherms are useful for estimating the total amount of adsorbent needed to adsorb a required amount of adsorbate from the solution.

Freundlich isotherm points a heterogeneous surface energy system and describes the adsorption process as "the ratio of the amount of solute adsorbed onto a given amount of adsorbent to the concentration of the solute in the solution is not constant at different concentrations". The logarithmic form of Freundlich model is given by the equation 5:

 $\log qe = \log Kf + \frac{1}{n}\log Ce \quad (5)$

where qe is the amount adsorbed and K_F and n are Freundlich constants related to the adsorption capacity and adsorption intensity, respectively. When log qe was plotted against log Ce, slope was obtained 1/n, were calculated from the slope and intercept of the plot log qe versus log Ce (shown in Fig. 7). K_F represents the amount of solute adsorbed at an equilibrium concentration of unity and thus is a measure of the adsorption capacity of the material, while n reflects the adsorption intensity (Table 1).

Another widely used equation in adsorption processes is the Langmuir equation. Langmuir model is represented by the following equation 6:

$$\frac{Ce}{qe} = \frac{1}{Q^{\circ}b} + \frac{Ce}{Q^{\circ}} \tag{6}$$

where Q^{o} is the amount of adsorbate at complete monolayer coverage (mg/g), which gives the maximum sorption capacity of sorbent and b (mL/mg) is the Langmuir isotherm constant that relates to the energy of adsorption calculated from the slope and intercept of the plot Ce/qe vs. Ce (shown in Fig. 8). The Langmuir constants Q^{o} and b are calculated and the values of these constants are tabulated for the highest R^{2} value in the Table 1.



Figure 7. Freundlich isotherm graph of the adsorption assay of β -glucosidase on MIP and NIP hydrogels.



Figure 8. Langmuir isotherm graph of the adsorption assay of β -glucosidase on MIP and NIP hydrogels.

When R^2 values of adsorption models were compared, the adsorption of β -glucosidase on MIP and NIP hydrogels was fitted to the Freundlich equation ($R^2 > 0.87$) and Langmuir

equation ($R^2 > 0.86$) respectively. This result demonstrates that the adsorption of β -glucosidase on MIP hydrogel can be characterized by multi-site adsorption isotherm. However adsorption of β -glucosidase on NIP hydrogel can better fit to Langmuir isotherm model suggesting that this adsorption is better to be described as a monolayer adsorption.

Table 1. Langmuir and Freundlich isotherm constants for adsorption of β -glucosidase on MIP and NIP hydrogels

| Langmuir Isotherms | | Freundlich Isotherms | |
|-------------------------------|-------------------------------|------------------------------|------------------------------|
| MIP | NIP | MIP | NIP |
| Q _{max} = 7.2 | Q _{max} =4.6 | K _F =0.16 | K _F =0.26 |
| mg/g | mg/g | mg/g | mg/g |
| b =0.0034 | b =0.0056 | 1/n= 0.55 | 1/n= 0.42 |
| L/mg | L/mg | | |
| $R_{L}=0.105$ | R _L = 0.066 | | |
| R²=0.733 | R²=0.864 | R ² =0.879 | R ² =0.835 |

Dimensionless separation factor R_L is a used to analyze the adsorption process. When R_L value is between 0 and 1 or higher than 1, this means the adsorption process is favorable or unfavorable respectively. If R_L equals to 1 or 0, it shows the adsorption process linear or irreversible respectively. ³⁵ R_L can be expressed as:

$R_L = 1/(1+bCo)$ (7)

Where b is the Langmuir constant and C_0 is the initial β glucosidase concentration (mg L⁻¹). Calculated R_L values for the adsorption of the enzyme onto MIP and NIP hydrogels were 0.105 and 0.066 respectively, showing that the adsorption process was favorable.

3.5 Kinetic parameters

Adsorption kinetics has an inseparable relationship with adsorption efficiency. The adsorption kinetic was investigated to measure the required equilibrium time for adsorption of β -glucosidase onto imprinted hydrogel. The adsorption kinetics of 2.5 mg/mL β -glucosidase solution at pH 7.0 was examined as a function of imprinted and non-imprinted hydrogels. In order to study the adsorption mechanism and verify the rate-determining step, pseudo-first-order and pseudo-second-order were utilized to fit the adsorption kinetics data. The nonlinear form of the mentioned two model equations can be expressed by equation (7) and (8), respectively:

$$\log(qe - qt) = \log qe - \frac{k_1}{2.303}t$$
 (7)

qe and qt indicate the amount of protein adsorbed at equilibrium and at a specific time (mg/g) and $k_1 (min^{-1})$ is the first-order rate constant. First-order rate constant k_1 was

calculated from the slope value of the linear plot of log (qeqt) vs. t (Figure 9).

$$\frac{t}{qt} = \frac{1}{k_2 q e^2} + \frac{t}{qt} \tag{8}$$

where qe (mg/g) is the amount of protein adsorbed at equilibrium, qt (mg/g) is the amount of protein on hydrogels at time. t and $k_2 [g/(mg min)]$ is the rate constant of pseudosecond-order adsorption. The values of $1/(k_2 q e)^2$ and 1/q eare derived experimentally from the intercept and slope of the linear plots of t/qt versus t, which eventually leads to values of k₂ and qe (Fig. 10). Relationship existing between experimental data and pseudo-second-order model and the correlation coefficient (R^2) in Table 2 suggest a strong relationship between the parameters and also explain that the adsorption process of enzyme both followed pseudosecond-order kinetics. The theoretical qe values were very well correlated with the experimental data in the case of pseudo-second-order kinetics. This also indicates that the adsorption process of the enzyme is controlled by chemisorption due to the several interactions between the enzyme and the functional groups of MIP and NIP hydrogels.



Figure 9. Pseudo-first-order model of the adsorption assay of β -glucosidase on MIP and NIP hydrogels.



Figure 10. Pseudo-second-order model of the adsorption assay of β -glucosidase on MIP and NIP hydrogels.

Table 2. Kinetic Models

| Pseudo-first-order | | Pseudo-second-order | |
|------------------------------|----------------------------|--------------------------------|--------------------------------|
| model | | model | |
| MID | NUD | MID | |
| MIP | NIP | MIP | NIP |
| R ² =0.661 | R²=0.840 | R ² =0.992 | R ² =0.920 |
| $q_{e} = 2.76$ | $q_{e} = 2.63$ | $q_{e}=4.36$ | q _e = 2.94 |
| mg/g | mg/g | mg/g | mg/g |
| $k_1 = 0.0437$ | $k_1 = 0.0392$ | k ₂ = 0.0175 | k ₂ = 0.0137 |
| min ⁻¹ | min ⁻¹ | g/mg.min. | g/mg.min. |

3.6 SDS-PAGE analysis

The SDS-PAGE analysis is shown in Figure 11. Lane i and h present the standard protein mixture and pure β -glucosidase. Dialysis fraction (e) was applied on MIP and NIP hydrogels and then desorption of the enzyme from hydrogels was done using 50 mM pH 5.0 citrate buffer. Desorption fractions of MIP and NIP hydrogels are seen in lane (b) and (a) respectively. Only the band of desorbed enzyme from MIP hydrogel could be easily seen. The SDS-PAGE analysis suggests that MIP hydrogel could specifically absorb β -glucosidase from the dialysis fraction. These results show that MIP hydrogels could be use as an affinity matrix in enzyme purification steps.



Figure 11 Results of SDS-PAGE analysis a) desorption of enzyme from NIP b) Desorption of enzyme from MIP c) unbound enzyme (MIP hydrogel) (10 fold dilution), d) unbound enzyme (MIP hydrogel) e) Dialysis fraction f) Acetone precipitation (5 fold dilution g) Acetone precipitation, h) Pure β -glucosidase i) protein molecular weight markers

4. Conclusions

 β -Glucosidase imprinted hydrogel was successfully synthesized in the presence of monomer acrylamide and crosslinker N,N'methylenebisacrylamide. Proteins are very complex and possess many potential recognition sites at their surface, such as charged amino acids and hydrophobic/hydrophilic regions. Molecular size and shape memory effect are also the major factors affecting the imprinting formation and template recognition. The imprinted hydrogel exhibits significant recognition property and selectivity to target β -glucosidase. The easy preparation, and high selectivity of β -glucosidase imprinted hydrogel exhibit its potential application in β -glucosidase separation.

Acknowledgment

This work is supported by Scientific Research Project Office of Manisa Celal Bayar University (BAP 2017-003)

References

- Bhattacharyya R and Ray SK 2013. Kinetic and equilibrium modeling for adsorption of textile dyes in aqueous solutions by carboxymethyl cellulose/poly(acrylamide-co-hydroxyethyl methacrylate) semi-interpenetrating network hydrogel. Polym Eng Sci 53(11):2439–2453.
- Çelik A, Dinçer A, Aydemir T 2016. Characterization of β glucosidase immobilized on chitosan-multiwalled carbon nanotubes (MWCNTS) and their application on tea extracts for aroma enhancement. Int J Biol Macromol 89:406–414.
- Ergöçen Gözde. Purification and characterization of β -glucosidase from apricot (*Prunus Armeniaca*) seeds. Çukurova University, Institute of Natural and Applied Sciences, Department of Chemistry, Adana, 2013, 45. (M.Sc.).
- Ghasemzadeh N, Nyberg F, Hjerten S 2008. Highly selective artificial gel antibodies for detection and quantification of biomarkers in clinical samples. I. Spectrophotometric approach to design the calibration curve for the quantification. J Sep Sci 31(22):3945-3953.

- Gómez JM, Romero MD, Fernández TM, Díez E 2012. Immobilization of β -glucosidase in fixed bed reactor and evaluation of the enzymatic activity. Bioprocess Biosyst Eng 35:1399–1405.
- Grover AK, Macmurchie DD, Cushley RJ 1977. Studies on almond emulsin beta-D-glucosidase. I. Isolation and characterization of a bifunctional isozyme. Biochim Biophys Acta. 482(1):98-108.
- Hawkins DM, Derek S, Subrayal MR 2005. Investigation of protein imprinting in hydrogel-based molecularly imprinted polymers (HydroMIPs) Anal Chim Acta 542:61–65.
- Hjerten S, Liao JL, Nakazato K, Wang Y, Zamaratskaia G, Zhang HX 1997. Gels mimicking antibodies in their selective recognition of proteins. Chromatographia 44(5-6):227-234.
- Kempe M, Mosbach K 1994. Direct resolution of naproxen on a non-covalently molecularly imprinted chiral stationary phase J Chromatogr A 664 (2):276-279.
- Kimhi O, Bianco-Peled H 2007. Study of the interactions between protein-imprinted hydrogels and their templates, Langmuir 23, 6329-6335.
- Li Z, Day M, Ding J, Faid K 2005. Synthesis and Characterization of functional methacrylate copolymers and their application in molecular imprinting. Macromolecules 38: 2620-2625.
- Lv Y, Tan T, Svec F 2013. Molecular imprinting of proteins in polymers attached to the surface of nanomaterials for selective recognition of biomacromolecules, Biotechnol Adv 31:1172–1186.
- Nishino H, Huang CS, Shea KJ 2006. Selective protein capture by epitope imprinting. Angew Chem 118:2452 –2456.
- Pan J, Xue X, Wang J, Xie H, Wua Z 2009. Recognition property and preparation of Staphylococcus aureus protein Aimprinted polyacrylamide polymers by inverse-phase suspension and bulk polymerization. Polymer 50:2365– 2372.
- Pisarev OA, Polyakova IV 2018. Molecularly imprinted polymers based on methacrylic acid and ethyleneglycol dimethacrylate for L-lysine recognition. React Funcnt Polym 130:98-110.
- Tong D, Heényi C, Bikádi Z, Gao J, Hjertén S 2001. Some studies of the chromatographic properties of gels ('Artificial antibodies/receptors') for selective adsorption of proteins. Chromatographia 54(1):7-14.
- Verheyen E, Schillemans JP, Wijk M, Demeniex MA, Hennink WE, Nostrum CF 2011. Challenges for the effective molecular imprinting of proteins, Biomaterials 32:3008-3020
- Verma OP, Singh A, Singh N, Chaudhary O 2011. Isolation, purification and characterization of β-glucosidase from *Rauvolfia serpentina*. J Chem Eng Process Technol 2:5.
- Wanga DD, Gao D, Xu WJ, Li F, Yin MN, Fu QF, Xi ZN 2018. Magnetic molecularly imprinted polymer for the selective extraction of hesperetin from the dried pericarp of Citrus reticulata Blanco. Talanta 187:307-315.
- Zaidi SA 2018. Development of molecular imprinted polymers based strategies for the determination of Dopamine. Sensor Actuat B Chem 265:488-497.
- Zhao K, Chen T, Lin B, Cui W, Kan B, Yang N, Zhou X, Zhang X, Wei J 2015. Adsorption and recognition of protein molecular imprinted calcium alginate/polyacrylamide hydrogel film with good regeneration performance and high toughness. React Functt Polym 87:7–14.
- Zhao M, Chen X, Zhang H, Yan H, Zhang H 2014. Well-defined hydrophilic molecularly imprinted polymer microspheres for efficient molecular recognition in real biological samples by facile RAFT coupling chemistry. Biomacromolecules 15(5):1663-75.