Comparative adsorption of amylase, protease and lipase on ZnFe₂O₄: kinetics, isothermal and thermodynamics studies

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Abstract The role of enzyme engineering in biotechnology, biological and pharmaceutical process cannot be over emphasized. This study compared the adsorption of digestives enzymes; amylase, protease and lipase on to Zn-ferrite (ZnFe₂O₄). The metal ferrite was synthesized via a sol–gel technique and characterized with scanning electron microscopy (SEM), X-ray diffraction (XRD), Electron paramagnetic resonance (EPR) and Fourier transform infrared spectroscopy (FTIR). The adsorption was studied in a batch process and the data were subjected to kinetics and isotherm models. Characterization shows that the particle has a nanoporous structure, with pore sizes of about 5.4 nm and good magnetic properties. The FTIR data showed the presence of M–O bond, which is a characteristic of metal ferrites. The adsorption of the amylase, lipase and protease on ZnFe₂O₄ follow first-order kinetic model with rate constants increasing with concentration. The maximum adsorption capacities as revealed by the generalized adsorption isotherms are 7.20, 42.90 and 22.24 mg g⁻¹ for amylase, lipase and protease, respectively, with cooperative binding. The Dubinin–Radushkevich model gave the maximum adsorption energies, E of 3.74 kJ mol⁻¹ for amylase, 2.01 kJ mol⁻¹ for lipase and 1.51 kJ mol⁻¹ for the protease adsorption, showing that the process is physisorption dominated. The isotherms fit the adsorption data in the order of Freundlich > Generalized > Guggenheim–Anderson–de Boer > Tempkin isotherm > Dubinin–Radushkevich. Thermodynamic study revealed a spontaneous adsorption process with increased entropy. ZnFe₂O₄, therefore, is a very good adsorbent for the purification of enzymes and can be used as a supporter for enzymatic process that required immobilization of the enzymes.

Keywords Adsorption • Enzymes • Zn-ferrite • Kinetic and isotherm

Introduction

Adsorption of proteins on solid surfaces is of great importance to many applications in bionanotechnology. For instance, enzyme immobilizations on nanoparticles are important precursors for the design of bionanocatalysts, bionanosensors and many other bionanotechnological-based materials, taking into cognizance the large surface to volume ratio of nanoparticles (Nune et al. 2009; Umut 2013; McNamara and Tofail 2017). Surface functionalized nanoparticles of biomedical application are being designed to target a particular protein based on the vulnerability of protein to adsorption onto surfaces (Slocik and Naik 2010; Assarsson et al. 2016). Although, enzyme adsorption on the surface is a prerequisite for these important applications, reduced or total loss in activity may accompany such adsorption (MacCormack et al. 2012). Careful selection of immobilization strategies and adsorbent materials can increase the dexterity of the adsorbed enzymes to work in a much broader pH and temperature range with higher thermal stability than the native ones (Xū et al. 2014). Immobilized enzymes find applications in processes requiring mechanical strength, microbial resistance, thermostability, chemical durability, chemical functionality,
low cost, hydrophilicity, regenerability and high capacity of enzyme (Burns 1976). Other advantages include; choice of batch or continuous processes, rapid termination of reactions, controlled product formation, ease of enzyme separation from the reaction mixture and adaptability to various engineering techniques (Bornhorst and Falke 2000; Kumar and Sharma 2015). Although these standard procedures are well established, their inability to cope with the samples containing particulate material in the early stages of the isolation/purification process is a big disadvantage. Since suspended solid and fouling components are present in the sample at this stage. In the cases of magnetic affinity, ion-exchange, hydrophobic or adsorption batch separation processes, applications of magnetically stabilized fluidized beds or magnetically modified two-phase systems have shown their advantage (Safarik and Safarikova 2004). The advantages of magnetic separation include; its simplicity with only few handling steps, inexpensive, inertness to the target peptides, which can also be used for the concentration of the peptides instead of ultrafiltration and precipitation. Moreover, the power and efficiency of magnetic separation procedures is useful at large-scale operations (Sarmiento et al. 2015). Compared with other techniques in enzyme immobilization/purification, adsorption has been considered to be the best due to its higher commercial potential, its simplicity and ability to retain high catalytic activity. Also, adsorption method offers the reusability of expensive supports after inactivation of immobilized enzyme (Gooding and Hall 1996; Yildiz and Gür 2007).

In this study, ZnFe$_2$O$_4$ was synthesized via a sol–gel technique and characterized with scanning electron microscopy (SEM), electron diffraction analysis (EDAX), X-ray diffraction (XRD), Electron paramagnetic resonance (EPR) and Fourier transform infrared spectroscopy (FTIR). It was used for the adsorptions of 3 extremoenzymes (α-amylase, lipase and protease) and their industrial application potentials and the fact that their enzymatic activities increase with purity (Robinson 2015). The kinetics data from their adsorptions studies were subjected to pseudo first order, second order, elovich and intraparticle diffusion kinetic models. Similarly, data from equilibrium studies were analyzed with Guggenheim–Anderson–de Boer (GAB), generalized adsorption isotherms, Freudlich, Tempkin and Dubinin–Raduskevithch (D–R) adsorption isotherm models. The temperature dependent adsorption data were used for the estimation of the thermodynamic parameters.

**Materials and methods**

**Materials**

All chemical used in the study were reagent grade, they were used without further purification. Ferric nitrate hexahydrate (Fe(NO$_3$)$_3$·9H$_2$O) and Zinc nitrate hexahydrate (Zn(NO$_3$)$_2$·6H$_2$O) were purchased from Sigma Aldrich, while citric acid and ammonium were from ACS reagents. All other reagents were of analytical grade and Milli-Q water was used for all the experiments.

**Preparation of ZnFe$_2$O$_4$ nanoparticle**

Zinc ferrite (ZnFe$_2$O$_4$) was synthesized via a sol–gel technique by weighing accurately 0.29748 g of Zn(NO$_3$)$_2$·6H$_2$O and 0.80799 g of Fe(NO$_3$)$_3$·9H$_2$O and dissolved in 10 ml Milli Q water. The solutions of the salt were mixed and stirred at 50 °C for 12 h. Citric acid was added as a chelating agent and the mixtures were allowed to evaporate at 90 °C until a dark brown sol–gel was formed. A sudden increase in temperature led to the combustion resulting into the formation of ZnFe$_2$O$_4$ nanoparticles. The as-prepared nanoparticles were then calcinated for 4 h at 800 °C. The dried material was characterized by different physicochemical techniques.

Surface morphology and elemental composition of the material was analyzed using scanning electron microscopy (SEM) [VEGA3 TESCAN], X-ray diffraction (XRD) data were collected using a PAN Analytical X’Pert PRO X-ray diffractometer with Cu Kα radiation (λ = 1.5418Å). Fourier transform infrared (FT-IR) spectra were recorded from 400 to 4000 cm$^{-1}$ in TENSOR 27 spectrometer (Bruker, Germany) using KBr pellet technique while the magnetic property were analyzed with BioSpin ESR EMX-8/2.7 system (Bruker, Germany).

**Adsorption and kinetic studies of the enzymes**

Crude enzymes were obtained from Microbiology Laboratory, Federal University of Agriculture Abeokuta, Nigeria. The enzymes were produced from *Aspergillus flavus* PW2961 strain obtained from the culture collection unit of the laboratory. Adsorptions of the enzymes (amylase, lipase and protease) onto the surface of ZnFe$_2$O$_4$ were carried out in a 10 ml vial by adding 5 ml of different concentrations (1.0–20.0 mg/L) of the crude enzyme to 0.2 g of the nanoparticle at room temperature and optimum activity pH (amylase 7.0, protease, 5 and lipase, 6.7). The final suspension was mixed for 30 s to disperse the nanoparticles and subsequently shaken for 2 h in an orbital shaker at 150 rpm. The adsorbed protein with nanoparticles
was separated from the mixture using an external magnetic field and the supernatant was analyzed for the protein content. The difference in protein concentration compared with the blank was the result of enzyme adsorption on to the adsorbent. Adsorption kinetics was studied by determining the amount of free protein in the supernatant by repeating the procedure above at different contact time. The amounts of enzyme adsorbed at time \( t \), \( Q_t \) (mg g\(^{-1}\)) and at equilibrium \( Q_e \) (mg g\(^{-1}\)) were calculated using Eqs. 1 and 2 below:

\[
Q_t = \frac{(C_o - C_t)V}{W} \tag{1}
\]

\[
Q_e = \frac{(C_o - C_e)V}{W} \tag{2}
\]

where \( C_o \) (mg L\(^{-1}\)) is the initial concentration and \( C_t \) (mg L\(^{-1}\)) is the concentration of the crude enzyme at time \( t \) in the liquid-phase. \( C_e \) (mg L\(^{-1}\)) is the concentration of the enzyme in the supernatant at equilibrium, \( V \) is the volume of the solution (L), and \( W \) is the mass of ZnFe\(_2\)O\(_4\).

To investigate the mechanisms of the adsorption process, pseudo-first order, pseudo-second order, Elovich kinetic and intraparticle diffusion models (Eqs. 3–6) were applied to describe the kinetics of the enzymes adsorption. For all Eqs. 3–6, \( Q_e \) is the amount of enzyme in mg g\(^{-1}\) adsorbed at equilibrium while \( k_1 \) and \( k_2 \) are the rate constant, \( K_d \) is intraparticle diffusion constant and \( C_l \) measures the thickness of the layer. The Elovich parameters \( \alpha \) and \( \beta \) are initial adsorption rate (mg g\(^{-1}\) min\(^{-1}\)) and the adsorption constant (g mg\(^{-1}\)) (Weber and Morris 1963; Lagergren 1996; Özacar and Şengil 2005; Adeogun and Balakrishnan 2016).

\[
Q_t = Q_e(1 - e^{-kt}) \tag{3}
\]

\[
Q_t = \frac{k_2Q_t^2}{1 + k_2Q_t} \tag{4}
\]

\[
Q_t = \frac{1}{\beta \delta} \ln(q_\delta \beta \delta \times t) \tag{5}
\]

\[
Q_t = k_d \times t^{0.5} + C_i \tag{6}
\]

Equilibrium data from the adsorption studies were also subjected to the Guggenheim–Anderson–de Boer (GAB), generalized adsorption isotherms, Freundlich, Tempkin and Dubinin–Raduskevich (D–R) adsorption isotherm models (Kargi and Ozmihci 2004; Crini and Peindy 2006; Zheng et al. 2008; Adeogun and Balakrishnan 2016) represented by Eqs. 7–10).

GAB adsorption model proposed a multilayer adsorption with addition that the state of the adsorbate in the other layers being the same but different from the first layer. The model is a combination of two distinct adsorption states, i.e., equivalent adsorption site where the adsorbate bind strongly with the possibility of the occupied site successively adsorbed weakly to the adsorbate. \( L_s \) and \( K_s \) are, respectively, the weak and strong state adsorption constants, while \( Q_o \) is the maximum adsorption in the inner wall of the adsorbent. This model will reduce to Langmuir equation for value \( K_s \) = 0 (Meissner et al. 2015). Generalized adsorption isotherm model is represented by Eq. 8, the model take into account the saturation of the binding site and cooperativity in the sites of adsorption. The value of \( m > 1 \) means an increase in affinity for other adsorbate molecule upon binding of adsorbate to one site, \( m < 1 \) mean binding in one site reduces affinity for others. Cooperative binding constant, \( m = 1 \) means independent binding of the adsorbent to available sites. \( K_G \) is the saturation constant in mg L\(^{-1}\) while \( Q_m \) is the maximum adsorption capacity (Kargi and Ozmihci, 2004). Freundlich isotherm described the heterogeneity in the surface. The \( K_f \) and \( n \) of Freundlich isotherm model (Eq. 9) are isotherm parameters characterizing adsorption capacity and intensity, respectively. Tempkin isotherm suggested a linear decrease in surface energy upon adsorption, taking into account the adsorbent/adsorbate interactions. Tempkin isotherm constants \( a_T \) and \( b_T \) in Eq. 10 are equilibrium binding constant (L/g).

\[
Q_{eq} = \frac{Q_oL_KC_e}{(1 + L_KE - K_sC_e)(1 - K_sC_e)} \tag{7}
\]

\[
Q_{eq} = \frac{Q_oC_m^m}{(K_G + C_e)^n} \tag{8}
\]

\[
Q_{eq} = K_FC_e^{1/n} \tag{9}
\]

\[
Q_e = \frac{RT}{b_T} \ln a_T C_e \tag{10}
\]

\[
Q_v = Q_v \exp(-\beta \varepsilon^2) \tag{11}
\]

The D–R sorption isotherm is based on ideal assumptions such as equipotient of the sorption sites, absence of stoic hindrance between adsorbed and incoming particles and surface homogeneity on microscopic level. D–R isotherm is represented by Eq. 11, where \( Q_m \) is the theoretical saturation capacity (mol g\(^{-1}\)), \( \beta \) is a constant related to the mean free energy of adsorption per mole of the adsorbate (mol\(^2\) J\(^{-1}\)), and \( \varepsilon \) is the Polanyi potential given by the relation; \( \varepsilon = RT \ln \left(1 + \frac{1}{C_e}\right) \). Ce is the equilibrium concentration of enzyme (mg L\(^{-1}\)), \( R \) (J mol\(^{-1}\) K\(^{-1}\)) is the gas constant and \( T \) (K) is the absolute temperature. The constant \( \beta \) gives an idea about the mean free energy \( E \) (kJ mol\(^{-1}\)) of adsorption per molecule of the adsorbate when it is transferred to the surface of the solid from relationship \( E = (2\beta)^{-0.5} \). If the magnitude of \( E \) is between 8 and 16 kJ mol\(^{-1}\), the process is chemisorption, while for values of \( E < 8 \) kJ mol\(^{-1}\) suggests a physical process. Non-linear regression analysis
method was used to obtain the least square fit for all the models, using a program written on Micro Math Scientist 3.0 software (Salt Lake City, Utah).

Statistical test

The acceptability and hence the best-fit of the kinetic data were based on the square of the correlation coefficients $R^2$ and the percentage error function which measures the differences (% SSE) in the experimental data and the values predicted by the models. The validity of each model was determined by the sum of error squares (SSE, %) given by:

$$%SSE = \sqrt{\frac{(Q_{exp} - Q_{cal})/Q_{exp})^2}{N - 1}} \times 100$$

where $Q_{exp}$ is the experimental data, $Q_{cal}$ is the calculated data, $N$ is the number of data points. The higher is the value of $R^2$ and the lower the value of % SSE, the better fitted the data.
data. A model is adjudged best-fit and selected based on statistical parameters.

Results

Characterizations of ZnFe$_2$O$_4$

The scanning electron micrograph and energy diffraction are presented in Fig. 1a–c. Figure 2 shows the typical XRD patterns of ZnFe$_2$O$_4$. The EDAX analysis showed that the surface is mainly constituted by the elements in the desired ratio. The EPR spectra of ZnFe$_2$O$_4$ were obtained at 9.859876 GHz at room temperature to determine its magnetic properties. EPR study of ZnFe$_2$O$_4$ is presented in Fig. 3 and its parameters were determined using $g = \frac{h}{m}$. Figure 4 shows FT-IR spectra of the ZnFe$_2$O$_4$ before (a) and after (b) the adsorption of the enzyme on the surface of ZnFe$_2$O$_4$.

Kinetics and mechanism of adsorption

The adsorption kinetic data of α-amylase, lipase and protease enzymes on ZnFe$_2$O$_4$ were subjected to kinetic model given by the Eqs. 3–6, Figs. 5, 6, 7 were obtained by the least square fit of the kinetic models using non-linear least square fit with the program written on Micro Math Scientist software (Salt Lake City, Utah) with the parameters shown in Table 1.

Adsorption isotherms

The amount of a substance adsorbed into the solid phase of adsorbent is related to the concentration of the substance in bulk solution at a particular temperature in equilibrium using the adsorption isotherm. Equilibrium adsorption data of α-amylase, lipase and protease enzymes on ZnFe$_2$O$_4$ were subjected to isotherm models given by the Eqs. 7–11, Fig. 8a–c were obtained by the least square fit of the models with the parameters shown in Table 2.

Thermodynamics of adsorption process

The thermodynamics parameters, i.e., $\Delta G^0$, $\Delta H^0$ and $\Delta S^0$ were estimated using the following relation:

$$\Delta G^0 = -RT \ln K_d$$

(13)

$$\ln K_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT}$$

(14)

The equilibrium constant, $K_d$, was estimated from the value of $Q/Q_e$ obtained at different temperature studies of equilibrium. Van’t Hoff plot of $\ln K_d$ against the reciprocal temperature is shown in Fig. 8.
of temperature (1/T), according to Eq. 14 produced straight lines (Fig. 9) with intercept as $\Delta S_0 / R$ and slope as $\Delta H_0 / R$.

**Discussion**

SEM analysis showed a well-defined porous structure of the ZnFe$_2$O$_4$ without a secondary phase. Figure 2 shows the typical XRD patterns of ZnFe$_2$O$_4$, all diffraction peaks compared favorably to cubic spinel (JCPDS No. 22-1012) structure of ZnFe$_2$O$_4$. No other impurity peaks were noted which is a clear indication of pure product. Using Debye–Scherrer equation, $D = \frac{k \lambda}{\beta \cos \theta}$ for the strongest peaks, the mean grain sizes of $\sim 5.42$ nm was obtained. The magnetic properties by Lorentzian fit of EPR data analysis gave resonance width ($\Delta H$) of 257.88 Gauss, resonance field of 3851 (Gauss) and the effective g-factor was estimated to be 2.06. These values compare favorably with ZnFe$_2$O$_4$ prepared by other methods the high g-factor is due to the presence of Zn$^{2+}$ in the ferrite structure. The FTIR analysis revealed the prominent absorption bands between 390 and 503 cm$^{-1}$ which slightly shifted to higher wave number upon adsorption could be attribute to Fe–O and Zn–O bonds vibrations. Upon adsorption, the intensity of the peak at 1690 cm$^{-1}$ reduced, which could be attributed to the interaction of the enzyme with nanoparticle.

Studying the adsorption kinetics and mechanism of the enzymes on ZnFe$_2$O$_4$ to estimate the optimum operational conditions and efficiency for full-scale processes is desirable. The kinetic of adsorption, revealed the rate constants

![Fig. 5 Kinetic fits for the adsorption of α-amylase on ZnFe$_2$O$_4$ a pseudo-first order model fits, b pseudo-second order model fits, c Elovich and d Intraparticle diffusion model fits](image-url)
while the mechanism reveals the steps involved in the adsorption processes. Figures 5, 6 and 7 showed that the quantity of enzyme adsorbed ($Q_t$) increases with time and enzyme concentrations. This trend is attributed to the gradual occupation of the empty sites as the adsorption progressed and become saturated at equilibrium. The first-order kinetic model best fitted the kinetic data with initial increase in rate constant as the initial enzyme concentration increases. Although average values of $R^2$ obtained for both first- and second-order kinetic model are almost the same, first order was adjudged as the best fit owing to the values of $Q_e$ obtained ($Q_e$ _calc), which are consistent with the experimental values ($Q_e$ _exp), statistical analysis with lower values of the %SSE is also in favour of first order model. The data fitted well with Elovich model ($R^2 > 0.9$) as shown in Table 1, increase in adsorption rate, $\alpha$ is noted with increasing enzyme concentrations. This may be as a result of increased probability of collision of enzyme with the adsorbent surface as the concentration increases. The desorption rate ($\beta$) on the other hand decreases with concentration for all the enzymes, since $\beta$ is linked to the distribution of activation energies of adsorption, therefore, adsorption of the enzymes at higher concentrations may be limited by energetic barriers Zhang and Stanforth (2005). The mechanisms of the adsorption of enzymes to ZnFe$_2$O$_4$ were investigated using intraparticle diffusion model. The intraparticle diffusion constant, $K_{di}$ increases the enzyme concentration, this is as result of the influence of increase in driving force and corresponding resistance of the surface boundary to the concentration gradient as the enzymes accesses the available sites on ZnFe$_2$O$_4$. The non-zero positive value of the intercepts $C_i$ showed that adsorption is not solely determined by intraparticle diffusion, rather mass transfer of the enzymes into the internal structure of

**Fig. 6** Kinetic fits for the adsorption of lipase on ZnFe$_2$O$_4$ a pseudo-first order model fits, b pseudo-second order model fits, c Elovich and d Intraparticle diffusion model fits
the adsorbent also played significant roles (Tuğet et al. 1998).

The Langmuir isotherm fit which proposed a monolayer adsorption on surfaces with identically homogeneous sites could not properly fit the equilibrium data, hence, the adsorption of related isotherms. It is obvious from the plot that the adsorption capacity increases initially, owing to the presence of active sites which got saturated with increase in enzymes concentrations. Guggenheim–Anderson–de Boer isotherm was used for the analysis of the adsorption of amylase, lipase and protease onto ZnFe$_2$O$_4$, respectively. The values of $K_s$ is far less than $L_k$ for all the enzymes, is a clear indication of predominance of strong adsorption of the enzymes onto the ZnFe$_2$O$_4$ structure than being weakly adsorbed, which means that the heat of adsorption of the second layer is identical to the higher layers, but heat of adsorption of the second layer and subsequent layers is less than the heat of fusion (Do 1998; Lemus 2011). The Generalized adsorption isotherm gives reasonable adsorption values of the maximum adsorption capacities because its assumptions are similar to that of Langmuir except that it includes cooperative binding of macromolecules with multiple binding sites. This isotherm gives maximum adsorption capacities of 7.02, 42.90 and 22.24 mg g$^{-1}$, respectively, for amylase, lipase and protease. The values of $m > 1$ obtained for all the enzymes suggested that binding of the enzymes were cooperative. Freundlich isotherm parameters, $n$ values of greater than 1 obtained for all the enzymes showed that the process of adsorption is highly favored. Tempkin isotherm parameters obtained showed that protease is adsorbed with the least energy, hence the maximum adsorption capacity obtained for this

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![Fig. 7 Kinetic fits for the adsorption of protease on ZnFe$_2$O$_4$](image-url) a pseudo-first order model fits, b pseudo-second order model fits, c Elovich and d Intraparticle diffusion model fits
Table 1 Parameters for the kinetic fits for the adsorption of amylase, lipase and protease on ZnFe$_2$O$_4$

| Pseudo First Order | Second order | Intraparticle diffusion | Elovich |
|--------------------|--------------|-------------------------|---------|
| mg/L $Q_{\text{eexp}}$ | $Q_{\text{e}}$ (mg g$^{-1}$) | $k_I$ (min$^{-1}$) | $R^2$ | %SSE | $Q_{\text{e}}$ (mg g$^{-1}$) | $k_2$ (g mg$^{-1}$ min$^{-1}$) | $R^2$ | %SSE | $k_{id}$ (mg g$^{-1}$ min$^{-0.5}$) | $C_{id}$ mg g$^{-1}$ | $R^2$ | $\alpha$ (mg (g min)$^{-1}$) | $\beta_{id}$ (g mg$^{-1}$) | $R^2$ |
| Amylase            |              |                         |        |        |                       |                       |        |        |                                 |               |       |                               |               |       |
| 1.0                | 0.163        | 0.205                   | 0.014  | 0.991  | 0.025                  | 0.313                 | 0.031  | 0.969  | 0.087                  | 0.014          | 0.006 | 0.947                          | 0.006          | 12.956 | 0.948                          |
| 2.0                | 0.559        | 0.625                   | 0.020  | 0.998  | 0.038                  | 0.863                 | 0.019  | 0.990  | 0.175                  | 0.053          | 0.001 | 0.979                          | 0.026          | 4.621  | 0.964                          |
| 2.5                | 1.126        | 1.113                   | 0.035  | 0.998  | 0.008                  | 1.325                 | 0.032  | 0.998  | 0.115                  | 0.104          | 0.072 | 0.960                          | 0.157          | 3.830  | 0.994                          |
| 3.0                | 2.097        | 2.037                   | 0.040  | 0.996  | 0.034                  | 2.376                 | 0.022  | 0.994  | 0.161                  | 0.193          | 0.156 | 0.945                          | 0.426          | 2.310  | 0.968                          |
| 4.0                | 3.299        | 3.235                   | 0.041  | 0.998  | 0.037                  | 3.747                 | 0.015  | 0.998  | 0.259                  | 0.307          | 0.264 | 0.939                          | 0.790          | 1.510  | 0.998                          |
| Lipase             |              |                         |        |        |                       |                       |        |        |                                 |               |       |                               |               |       |
| 5.0                | 0.409        | 0.741                   | 0.007  | 0.858  | 0.192                  | 1.342                 | 0.003  | 0.856  | 0.539                  | 0.030          | 0.044 | 0.796                          | 0.012          | 3.777  | 0.871                          |
| 7.0                | 1.008        | 1.455                   | 0.011  | 0.912  | 0.258                  | 2.412                 | 0.003  | 0.908  | 0.811                  | 0.081          | 0.074 | 0.867                          | 0.032          | 1.753  | 0.887                          |
| 10.0               | 2.040        | 2.337                   | 0.019  | 0.982  | 0.172                  | 3.304                 | 0.004  | 0.976  | 0.730                  | 0.190          | 0.022 | 0.961                          | 0.087          | 1.179  | 0.930                          |
| 12.0               | 3.475        | 3.884                   | 0.020  | 0.989  | 0.236                  | 5.356                 | 0.003  | 0.984  | 1.086                  | 0.331          | 0.001 | 0.971                          | 0.162          | 0.737  | 0.943                          |
| 15.0               | 5.472        | 6.026                   | 0.023  | 0.977  | 0.320                  | 8.086                 | 0.002  | 0.969  | 1.509                  | 0.531          | 0.064 | 0.948                          | 0.285          | 0.485  | 0.868                          |
| Protease           |              |                         |        |        |                       |                       |        |        |                                 |               |       |                               |               |       |
| 5.0                | 0.766        | 0.741                   | 0.055  | 0.994  | 0.014                  | 0.823                 | 0.111  | 0.998  | 0.033                  | 0.071          | 0.081 | 0.897                          | 0.877          | 9.002  | 0.989                          |
| 8.0                | 1.891        | 1.789                   | 0.065  | 0.986  | 0.059                  | 1.958                 | 0.061  | 0.993  | 0.039                  | 0.174          | 0.216 | 0.879                          | 6.415          | 4.387  | 0.845                          |
| 10.0               | 3.976        | 3.867                   | 0.060  | 0.996  | 0.063                  | 4.234                 | 0.026  | 0.999  | 0.149                  | 0.373          | 0.461 | 0.881                          | 10.886         | 1.980  | 0.998                          |
| 15.0               | 7.206        | 6.978                   | 0.064  | 0.995  | 0.132                  | 7.584                 | 0.016  | 0.999  | 0.218                  | 0.674          | 0.869 | 0.872                          | 36.913         | 1.196  | 0.988                          |
| 20.0               | 11.111       | 10.924                  | 0.091  | 0.999  | 0.108                  | 11.373                | 0.025  | 0.999  | 0.151                  | 1.048          | 1.669 | 0.817                          | 202.693        | 1.572  | 0.931                          |
adsorbent. Dubinin–Radushkevich model gave theoretical saturation capacities values $Q$, of 1.62, 9.99 and 8.45 mg g$^{-1}$, respectively, for amylase, lipase and protease, while the maximum adsorption energies $E$, ranges between 1.51 and 3.74 kJ mol$^{-1}$, showing that the process is physisorption dominated process. The overall comparison of the Isotherms using the average values of $R^2$ shows that the isotherm fit are in the order Freundlich > Generalized > Guggenheim–Anderson–de Boer > Tempkin isotherm > Dubinin–Radushkevich.

The free energy change, $\Delta G$ is obtained from Eqs. (13, 14) according to the Van’t Hoff linear plots of $\ln K_d$ versus $1/T$ plot in Fig. 9. The thermodynamic parameters are presented in Table 3. From Fig. 9 and the parameters presented in the Table, it is found that the negative value of $\Delta G$ indicates the spontaneous nature of adsorption. Positive value of enthalpy change indicates that the adsorption process is endothermic in nature, while the entropy changes, $\Delta S$ showed increased randomness at the adsorbate–adsorbent inter phase during the adsorption process.

**Conclusion**

ZnFe$_2$O$_4$ nanoparticles was successfully synthesized and confirmed by characterization. The adsorption of the amylase, lipase and protease on ZnFe$_2$O$_4$ follow first order kinetic model when compared with second order kinetic model. The adsorption energy increases with concentration as shown by the Elovich model, while the intraparticle diffusion model suggested mass transfer as one of the factors affecting adsorption. The maximum adsorption capacities as revealed by the generalized adsorption isotherms are 409.03, 103.56 and 73.67 mg g$^{-1}$, respectively, for amylase, lipase and protease, respectively, with cooperative binding. The Dubinin–Radushkevich model gave
the maximum adsorption energies, $E$, ranges between 0.162 and 0.567 kJ mol$^{-1}$ showing that the process is physisorption dominated process. Thermodynamic study revealed a spontaneous adsorption process with increase in entropy. ZnFe$_2$O$_4$, therefore, is a very good adsorbent for the purification of enzymes and can be used as a support for enzymatic process that required immobilization of the enzymes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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