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α -amylase immobilized composite cryogels: Some studies on kinetic and adsorption factors

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Abstract

Stability of enzymes is significant factor for their industrial feasibility. α -amylase is an important enzyme for some industries i.e., textile, food, paper and pharmaceuticals.. Pumice particles (PPa) are non-toxic, natural, low-cost alternative adsorbents with high adsorption capacity. In this study, Cu^{2+} ions were attached onto pumice particles (Cu^{2+} -APPa). Then, Cu^{2+} -APPa embedded composite cryogel was synthesized (Cu^{2+} -APPaC) via polymerization of gel-forming agents at minus temperatures. Characterization studies of the Cu^{2+} -APPaC cryogel column was performed by X-ray fluorescence spectrometer (XRF), scanning electron microscopy (SEM), and Brunauer, Emmett, Teller (BET) apparatus. The experiments were carried out in a continuous column system. α -amylase was adsorbed onto Cu^{2+} -APPaC cryogel with maximum amount of 858.7 mg/g particles at pH 4.0. Effects of pH and temperature on the activity profiles of the free and the immobilized α -amylase were investigated, and results indicate that immobilization did not alter the optimum pH and temperature values. k_{cat} value of the immobilized α -amylase is higher than that of the free α -amylase while K_M value increases by immobilization. Storage and operational stabilities of the free and the immobilized α -amylase were determined for 35 days and for 20 runs, respectively.

Keywords: Protein Adsorption, IMAC, α -Amylase, Composite cryogel, Bead embedding

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1. Introduction

Enzymes are known as biological catalysts that facilitate complex chemical processes within optimum conditions. α -amylase (EC 3.2.1.1) is a significant enzyme, especially in textile [1], food processing [2], paper pulping [3] and pharmaceutical industries [4]. Amylases have been a source of inspiration for some research areas regarding their applications in the food and fermentation business lines. And also, they are used for hydrolysis of starch and the manufacturing of oligosaccharides [5]. According to the information in the literature, it is supposed that enzyme production and application will reach up to 17.5 billion dollars in 2024 [6].

Application of free enzyme in bioprocessing has some negative sides such as leanness, instability and poor regain [7]. Enzyme purification technology is an important factor as these conditions can cause increased bioprocess expenses [8]. Immobilization of α -amylase on carriers appears the most appropriate method to acquire stable and repeated used forms of enzymes. In previous studies, immobilization of α -amylase on various supports such as magnetic nanoparticles [9], metal ion affinity adsorbent [10] microparticles [11], cellulose membrane [12], nanosphere [13], composite cryogels [14] and beads [15] has been conducted. Immobilization of enzymes on the support materials is very efficient option for overcoming the troubles such as instability, cost, sensibility and repeatability.

The affinity of the proteins to metal ions can provide facility for their purification and analysis [16]. Lately, immobilized metal affinity chromatography (IMAC) is intensely preferred for purifying biomolecules due to its properties such as low cost, large capacity for biomolecules and high chemical stability [17, 18]. This chromatographic technique relies on coordination between functional groups of surface reachable amino acids and chelated metal ions [19, 20].

Cryogels are formed by the polymerization of gelation agents at minus temperatures [21, 22]. They ensure some facilities such as little residence time because of short diffusion route, low-pressure drop etc. when compared to the stereotype techniques [23]. Nevertheless, low surface area of the supermacropores gives rise to low adsorption capacity [24]. Particle embedded cryogels have been taken attention of scientists because of the combination of significant properties of cryogels and embedded particles. Cryogels offer perfect mass flow with supermacroporous structures whereas pumice particles present high adsorption capacity with high surface area and ligand density [22].

The immobilization of enzymes has been conducted onto some solid surfaces in the literature such as carbon nanotube [25], activated carbon [26], nanozeolite [27], silica material [21] and pumice particle [23]. Pumice particles are natural sorbents that low have cost, non-toxic and high surface area for high adsorption capability when compared to artificial commercial adsorbents and other natural materials [28]. And also, they have chemical reactivity and hydrophilicity features too. Thanks to these properties, pumice particles were used as a natural adsorbents in this study.

To summarize all points described above, we developed a new kind of functional, inexpensive, composite cryogel column for immobilization of α -amylase. The Cu^{2+} -APPaC and Cu^{2+} -APPa were characterized by some equipments such as XRF, SEM and BET. Effects of pH, ionic strength, initial α -amylase concentration, temperature, and flow rate on α -amylase immobilization onto the Cu^{2+} -APPaC were studied. In order to take some decisions on the features of α -amylase immobilization, adsorption isotherms were examined. Moreover, the effects of pH and temperature on the activity profiles of free and immobilized amylase were identified. Kinetic parameters and thermal, operational and storage stabilities were also determined.

2. Materials and methods

2.1 Materials

The pumice particles were acquired from Pumice Research Centre (through Dr. Fatma Gurbuz), Süleyman Demirel University. 2-Hydroxyethyl methacrylate (HEMA) was bought from Fluka A.G (Buchs, Switzerland). *N,N'*-methylene-bis-acrylamide (MBAAm), *N,N,N',N'*-Tetramethylethylene-diamine (TEMED) and ammonium persulfate (APS) were ensured by Sigma (St. Louis, MO, USA). 3,5-dinitrosalicilic acid (DNSA), starch, sodium–potassium tartarate, and α -amylase were provided by Aldrich (Munich, Germany). Other chemicals were purchased at reagent grade from Merck AG (Darmstadt, Germany).

2.2 Preparation of natural pumice particles (PPa)

The natural pumice particles were obtained by pre-treatment for the elimination of substances that can be extracted and can influence surface area of natural the particles. For this aim, pumice particles were waited in acid (3.0 M HCl) and alkali (3.0 M NaOH) solutions for 24 hours. Additionally, a heating method was operated at 130 °C for 5 h for the elimination of

organic contaminants from the pore structures. Thus, the surface area of natural particles has been expanded. The treated particles were washed by using distilled water and dehydrated at 180 °C for 24 hours.

2.3 Attachment of Cu²⁺ ions to natural pumice particles

Attachment of Cu²⁺ ions to PPa was conducted (at room temperature, 2 h) with a Cu²⁺ solution (100 ppm, pH 5.0 tuned up with 0.01 M HCl). The amount of attached Cu²⁺ ions onto PPa were determined through beginning and last solutions of Cu²⁺ ion by a graphite furnace atomic absorption spectrometer (GFAAS, Analyst 800/PerkinElmer, Waltham, MA).

2.4 Organizing of Cu²⁺-attached pumice particles (Cu²⁺-APPa) embedded cryogel column

The polymerization of Cu²⁺-APPa embedded cryogel column is as follows: HEMA (6 mmol) as monomer and MMBAm (1 mmol) as cross-linker were dissolved with deionized water (14 mL). The N₂ atmosphere was passed through the mixture for 5 min to remove the dissolved oxygen. After 10 mg Cu²⁺-APPa was suspended in the polymerization solution, APS (10%, w/v) as the free radical producer and TEMED (20 μL) as the initiator were added immediately. Polymerization solution was filled into a plastic syringe (5 mL, i.d. 0.8 cm) and left in the refrigerator to freeze at -12 °C for 24 h. After completion of this period, Cu²⁺-APPa embedded column was taken out of refrigerator, thawed, and cleaned up many times with water.

2.5 Characterization of cryogel samples

The porosity symbolized with ϕ was figured out for the presentation of free-water level proportion in the Cu²⁺-APPaC cryogel sample by the volume. The saturation of a portion of composite cryogel sample was ensured by deionized water. A portion of cryogel was dipped in water of volume V_1 . For the total volume of measuring cylinder was defined as V_2 . The volume difference was calculated as in the following equation:

$$V_0 = V_2 - V_1 \quad (1)$$

After swelling the cryogel piece up with water, it was pressed by hand. And these pieces obtained from different events were weighed and noted as m_w and m_s in order. Then, the Eq. 2 was applied for determining of the porosity. Here “ ρ_w ” symbolizes the deionized water density.

$$\phi = (m_w - m_s) / \rho_w \times V_0 \times 100 \quad (2)$$

For the occurrence of fully dried cryogel, it was put in the oven (60 °C, 12-24 h) and “ m_d ” (dried cryogel mass) was figured out. The Eq. 3 was utilized for total water fraction (TWF):

$$TWF = (m_w - m_d) / \rho_w \times V_0 \times 100 \quad (3)$$

The textures of Cu^{2+} -APPaC and null cryogels were examined by scanning electron microscopy (SEM). The samples were swelled in distilled water. Later, they were left absolute (98%) ethanol to send away water molecules with alcohol ones in the pores. As the next step, the samples were placed into vacuum oven (at 50 °C) to get rid of alcohol from the sample without damaging to construction [29]. Subsequently, the dried samples were coated with gold-palladium (40:60) and investigated with JEOL JSM 5600 (Tokyo, Japan) for SEM analysis. The chemical composition of the pumice particles were studied by X-ray fluorescence spectrometer (XRF) (Thermo Scientific, Waltham, MA, ARL 9900). Specific surface area (m^2/g) of the pumice particles were also analysed through BET (Brunauer, Emmett, Teller) method by utilization of the adsorption of N_2 (Micromeritics, ASAP 2020, Norcross (Atlanta), GA).

2.6 Adsorption-desorption studies from aqueous solution

Adsorption experiments were conducted to show the influence of different experimental parameters such as pH, initial α -amylase concentration, ionic strength, flow rate, and temperature on α -amylase adsorption capacity. The studies on α -amylase adsorption onto Cu^{2+} -APPaC column was performed in a continuous system equipped with water jacket for temperature control. The Cu^{2+} -APPaC cryogel was equilibrated by using operated buffer before α -amylase adsorption solutions were sent to the performed column through a peristaltic pump (ALITEA, Sweden). Initial and final protein concentrations were identified at 280 nm with a UV-spectrophotometer (Thermo Scientific GENESYS 10S UV/Vis spectrophotometer). All adsorption experiments were conducted three times for statistical attitude. Elution stages were performed with 0.5 M NaCl at flow rate of 1.0 mL/min. The reusability of Cu^{2+} -APPaC column was controlled by using the same column for 35 adsorption-desorption cycles.

2.7 Activity studies for free and immobilized amylase

α -Amylase activity was identified by the method of Bernfeld [30]. The assay includes spectrophotometric measurement of maltose released from starch by reducing with 3,5-dinitrosalicylic acid (DNSA). Free and immobilized amylase are reacted with 1% starch solution

(0.1 mL) for 3 min. Enzyme reaction is stopped by the addition of assay solution containing sodium–potassium tartarate (100 mM), NaOH (400 mM) and, DNSA (4.4 mM). Reaction solution is incubated in water bath at 95 °C for 5 min and cooled fastly. Bidistilled water (2 mL) is added and the intensity of coloured complex is examined with a UV-spectrophotometer (Thermo Scientific GENESYS 10S UV/Vis spectrophotometer) at 540 nm. Various maltose solutions (0.1-1.5 mM) are used to form calibration curve for the determination of the released maltose amount. One amylase activity unit is defined as the amount of maltose released by mg amylase per min under specified conditions (25 °C and pH 6.9). Effects of pH and temperature on the free and immobilized amylase activity were investigated between pH 4.0 and pH 8.0 and, in the range of 5 and 45 °C. K_M (Michaelis constant) and V_{max} values were calculated from Lineweaver-Burk plot generated using different starch solutions (0.2% - 2.0%).

2.8 Thermal, storage and operational stabilities

The thermal stabilities of the free and immobilized α -amylase at the optimum temperature were analysed hourly for 6 h. The storage stabilities of the free and immobilized amylase were determined routinely for 35 days. The operational stability was determined by repeated amylase activity runs using the same amylase immobilized Cu^{2+} -APPaC column at 25 °C repeatedly. Amylase immobilized Cu^{2+} -APPaC column was washed with pH 5.0 acetate buffer between the activity runs.

3. Results and discussions

Fig. 1 demonstrates the SEM images of null (a) and Cu^{2+} -APPaC cryogels (b). It can be seen from the SEM images, Cu^{2+} -APPaC cryogel has extremely porous surface and spongy structures. Cu^{2+} -APPaC column showed higher α -amylase adsorption (mg/g) in comparison to the adsorption capacity of null column (mg/g). Null column have showed insufficient α -amylase adsorption due to the poor surface area. As presented in SEM photos, Cu^{2+} -APPaC were distributed regularly into cryogel column which is why this composite structure with high surface area serve for circulation of mobile phase, and increase the adsorption capacity due to the union of these binary structure.

Insert Fig. 1

Chemical combination of pumice particles was indicated by XRF. According to results of XRF characterization, pumice particles occur from oxides of Si (~70%), Al (~13%), K (~4%) and N (~3%), Fe (~1.5%), Ca (~1%), Mg (~0.5%), Ti (~0.2%), Mn (~0.1%).

The porosity measurement, ϕ , and the total water content, *TWC*, for Cu²⁺-APPaC cryogel was calculated respectively as 70.2% and 93.3% (v/v). Results showed that small pores of Cu²⁺-APPaC column have bound 23.1% of the total water while flowing liquid was not passing through the Cu²⁺-APPaC column. The large pores wherein the liquid following paths were occurred and formed 70.2% of the total pores, were filled with free water. The surface areas of PPa, null and composite cryogel were computed specifically by BET-method as well, and found to be 64.7, 36.2 and 54.8 m²/g, respectively. When null and composite cryogels are compared regarding specific surface areas, it is seen that PPs enhance it up to 51.4%. It is also noted that amount of chelation of Cu²⁺ ions on PPs was found as 1.46 mg Cu²⁺ ions/g PPs.

3.1 Adsorption-desorption studies from aqueous solution

The effects of pH, concentration, flow rate and ionic strength were carried out for the optimization of α -amylase adsorption onto Cu²⁺-APPaC column. pHs in the range of 3.0 to 6.0 were performed to see the pH effect, and the results were shown in Fig. 2a. The maximum adsorption capacity was occurred when the pH reached at 4.0. This situation can be explained as follows: α -amylase contains acidic amino acids (i.e., aspartate and glutamate) mostly since its isoelectric point is near the acidic region [14, 31]. It was noticed that IMAC matrix can act as a pseudo-anion exchanger even if there are no IMAC active amino acid residues (i.e., histidine, cysteine) near by the surface for metal ligand [32]. In IMAC procedure, the presence of aspartate and /or glutamate residues on the protein surface concludes electrostatic interaction. This phenomenon is called as mixed mode interactions [33]. Because of deprotonated form of pK_R 's of amino acid residues like aspartate at pH 4.0, a high adsorption was obtained at this pH.

To determine the influence of initial α -amylase concentration which changed between 0.1 and 4 mg/mL, both Cu²⁺-APPaC and non-Cu²⁺ incorporated APPaC columns were used for specific and nonspecific binding capacity tests. As shown from Fig. 2b, Cu²⁺-APPaC column adsorbed considerably much more α -amylase (858.7 mg/g) than non-Cu²⁺ incorporated APPaC column (42.9 mg/g) at 4 mg/mL α -amylase concentration.

Insert Fig. 2.

The flow rate is the another major parameter which has an effect over binding amount on adsorbent in a column system. The adsorption experiments were figured out in a definite range (0.5-3 mL/min). Maximum adsorption was achieved at the lowest flow rate (Fig. 3a). The rise of flow rate paved the way to a decline in adsorption. Interaction period has been shorter between protein and ligand by increasing of flow rate that's why α -amylase molecules have short time to make interaction with walls of column fitted by ligands [27].

To study the effect of ionic strength on the α -amylase adsorption on Cu^{2+} -APPaC column, the different NaCl concentrations (0-0.5 M) were implemented to column. As shown from Fig.3b, the rising of ionic strength has been ended up with low adsorption. Principally, this effect can be ascribed to electrostatic interactions. Increasing of NaCl concentration in the medium makes shield effect to the surface of adsorbent [34].

Insert Fig. 3

The reusability of Cu^{2+} -APPaC column had a satisfactory degree for next studies. In this study, over 95% of adsorbed α -amylase was desorbed by 0.5 M NaCl during a short time of 20 min. Adsorption-desorption stages were repeated 30 times by operating the same column, and a remarkable changing wasn't observed in cryogel construction.

3.2 Effect of pH and temperature on the activities of free and immobilized amylase

It is admitted that pH and temperature activity profiles are affected by enzyme immobilization. Therefore, the effect of pH and temperature on the free and immobilized amylase activity identified and depicted in Fig. 4.

Insert Fig. 4.

As seen in Fig. 4a, immobilization did not alter the optimum pH value and specified it as pH 5.0. Furthermore, the immobilized α -amylase indicated higher activity than that of the free amylase in pH region between pH 6.0 and 8.0. Though, the activity of immobilized α -amylase is determined lower as regards to the free α -amylase at pH 4.

α -amylase activity has increased between 5 and 35 °C and optimum temperatures for both the free and the immobilized α -amylase are designated as 35 °C (Fig. 4b). It is determined that the activity of the immobilized α -amylase is higher around 10% than that of free amylase at 5 and 25 °C while activity values are not statistically different at 15 °C. The maximum effect of immobilization on temperature profile is observed at 45 °C. The activity of immobilized α -amylase is 3-fold higher than that of the free α -amylase at 45 °C. Drastic decrease for the activity of free α -amylase at 45 °C may be explained by structural changes of the enzyme at high temperature values since effect of temperature indicates structural changes [35, 36].

3.3 Thermal, operational and storage stabilities

Thermostabilities of free and immobilized amylase were investigated for 6 hours and shown in Fig. 5a. Thermostability of the free and immobilized α -amylase differ after 1 hour. Relative activity values at the end of 6 hours are determined as 90.32% and 96.00% for the free and the immobilized α -amylase, respectively. Relative activity of free α -amylase diminish at about 10% while the activity loss of the immobilized α -amylase is statistically not significant.

For the determination of operational stability, α -amylase immobilized Cu^{2+} -APPaC column was performed to 20 α -amylase activity assays. After 20 activity runs, α -amylase immobilized Cu^{2+} -APPaC column conserved 97.16% of its initial activity. In the literature for a few studies, Mulko et al. determined similar kinetics for α -amylase immobilized nanoporous polyacrylamide-graphene oxide nanocomposites up to 5 cycles [37]. Mardani et al. showed that the α -amylase activity immobilized on chitosan-montmorillonite nanocomposite beads decreased about 47% after reusing 5 times [38]. α -amylase immobilized titania/lignin hybrid material showed 90% activity preservation after 5 cycles in the study of Klapiszewski et al. Nevertheless, 90% of its initial activity was consumed at the end of 20 cycles [39]. Antony and Mohanan determined that after 15 cycles for reuse, α -amylase immobilized on polypyrrole retained almost 50% of its initial activity [40]. Almulaiky et al. studied immobilization of α -amylase onto hydroxyapatite (HA) and hydroxyapatite-decorated ZrO_2 nanocomposite and indicated that after 10 repeated cycles, 46% and 70% of initial activities were conserved for HA and HA- ZrO_2 nanocomposite, respectively [41]. When comparing, it is seen that results obtained here showed the higher operational stability and multithreading feature of α -amylase immobilized Cu^{2+} -APPaC column.

The activity assays of the free and the immobilized α -amylase routinely for 35 days and results are given in Fig. 5b. At the end of 20 days, relative activity values are determined as 45.42% and 91.55% for the free and the immobilized α -amylase, respectively. At the end of 35 days, the immobilized α -amylase still indicates 45.54% of its initial activity as the free α -amylase used up its initial activity. This result proved that immobilization considerably enhanced the storage stability of α -amylase.

Insert Fig. 5.

3.4 Kinetic Parameters

Various starch solutions (0.2%-2%) were used for α -amylase activity assays, and V_{max} values were calculated as 1.67×10^5 and 2.5×10^5 $\mu\text{mol}/\text{min}$ for free and immobilized α -amylase, respectively. A 1.5-fold increase at maximum enzyme velocity is determined by immobilization. However, K_M values are calculated as 6.67 and 75 g/L for free and immobilized α -amylase, respectively. The notable increase at K_M value shows that affinity of the enzyme to its substrate decreased by immobilization. Turnover number, k_{cat} , is a helpful value to compare catalytic efficiency of the free and the immobilized enzyme [42]. k_{cat} value of the immobilized α -amylase is specified ($4.82 \times 10^8 \text{ min}^{-1}$) as higher than that of the free α -amylase ($5.46 \times 10^7 \text{ min}^{-1}$). The catalytic efficiency of α -amylase is increased since k_{cat} value expresses the number of converted substrate by the enzyme per unit time.

4. Conclusion

This paper reports a new method for design of composite cryogel column for enzyme immobilization with lots of advantages such as having high steadiness, simple preparation, excellent binding capability, low cost, time saving. Here, Cu^{2+} -APPaC column was synthesized through free radical polymerization. The pumice particles and composite cryogel were characterized by XRF, SEM, BET experiments. The maximum adsorption was occurred at pH 4.0 (858.7 mg/g particles). Moreover, the reusability tests were confirmed 30 adsorption-desorption cycles with 0.5 M NaCl for about 20 minutes by using the same column. Activity results indicate that the optimum pH and temperature values are not affected by immobilization and determined as pH 5.0 and 35 °C, respectively. V_{max} value of the immobilized α -amylase is 1.5-fold higher than that of the free α -amylase. The increase at k_{cat} value by immobilization shows the enhanced catalytic efficiency. Cu^{2+} -APPaC column was performed to 20 α -amylase activity assays to investigate the operational stability and considerably high percent of initial

activity (97.16%) is designated. Results for operational stability in this study are higher than that of in the literature. Therefore, it can be said that α -amylase immobilized Cu^{2+} -APPaC column with high operational stability is convenient for repeated use in industrial applications. Moreover, immobilization increases storage stability of α -amylase, and the immobilized α -amylase indicates 45.54% of its initial activity whereas the free α -amylase consumed all activity at the end of 35 days. Based on all the results from this experiment, it can be considered that this composite column prepared could be a source of inspiration for future enzyme studies.

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Authors' Contributions

Ömür Acet: investigation, writing-review & editing

Tülden İnanan: investigation, conceptualization, writing-review & editing

Burcu Önal Acet: Investigation

Emrah Dikici: Investigation.

Mehmet Odabaşı: conceptualization, writing-review & editing.

All the authors agreed to submit the manuscript.

Data Availability

Available on request.

Compliance with Ethical Standards

Consent to Participate

All the authors agreed to participate in the scientific work

Conflict of Interest

The authors report no declarations of interest.

Ethical Approval

Not applicable.

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Figure Legends

Fig. 1. SEM images of null (a) and Cu²⁺-APPaC cryogel (b)

Fig. 2. Effect of pH on α -amylase adsorption (Embedded Cu²⁺-APPa: 20 mg, α -amylase concentration: 1 mg/mL, Flow rate: 1 mL/min, T: 25 °C) (a); Effect of concentration on α -amylase adsorption (pH: 4.0, Embedded Cu²⁺-APPaC: 20 mg; Flow rate: 1 mL/min; T: 25 °C) (b)

Fig. 3. Effect of flow rate on α -amylase adsorption (pH 4.0, Concentration of α -amylase: 1 mg/mL, Embedded Cu²⁺-APPa: 20 mg, T: 25 °C) (a); Effect of ionic strength on α -amylase adsorption (pH 4.0, Concentration of α -amylase: 1 mg/mL, Embedded Cu²⁺-APPa: 20 mg, Flow rate: 1 mL/min; T: 25 °C) (b)

Fig. 4. Effect of pH on the free and immobilized amylase activities (Concentration of α -amylase: 1 mg/mL, Embedded Cu²⁺-APPa: 20 mg, T: 25 °C) (a); Effect of temperature on the free and immobilized amylase activities (Concentration of α -amylase: 1 mg/mL, Embedded Cu²⁺-APPa: 20 mg; pH:5.0) (b)

Fig. 5. Thermostability of the free and immobilized α -amylase (Concentration of α -amylase: 1 mg/mL, Embedded Cu²⁺-APPa: 20 mg, pH:5.0) (a); Storage stabilities of the free and immobilized α -amylase (Concentration of α -amylase: 1 mg/mL, Embedded Cu²⁺-APPa: 20 mg, pH:5.0, T: 4 °C) (b)

Figures

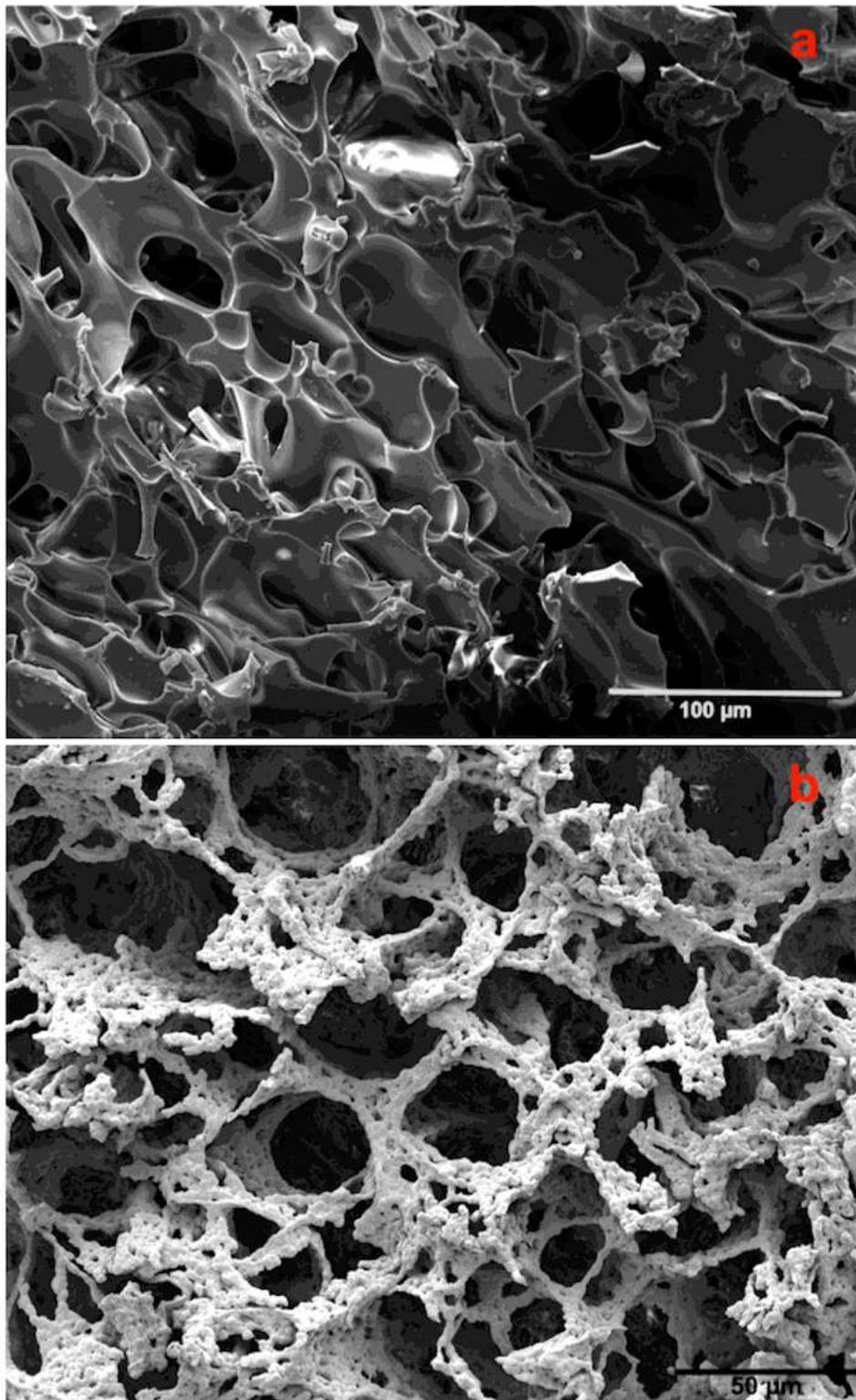


Figure 1

SEM images of null (a) and Cu²⁺-APPaC cryogel (b)

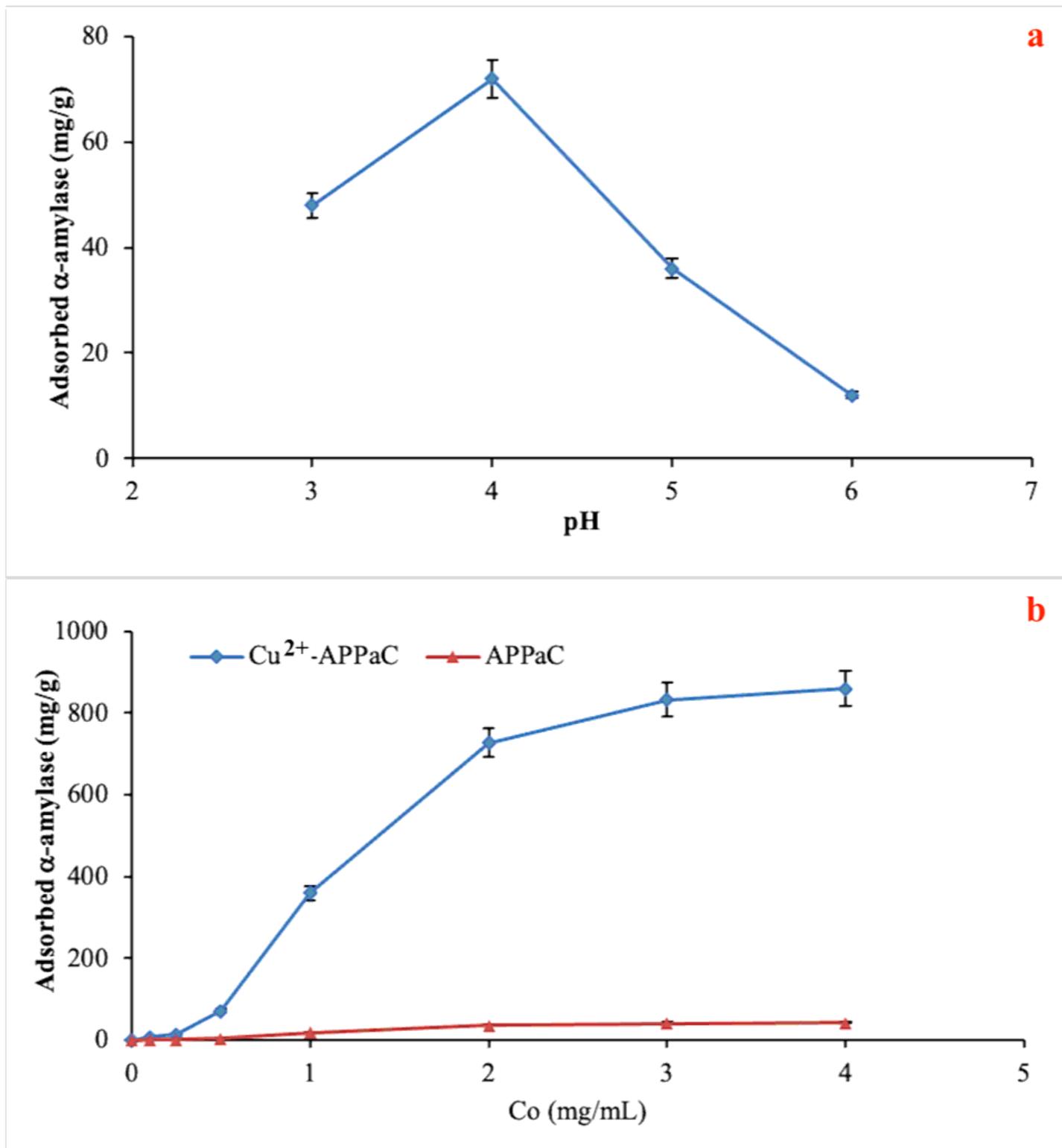


Figure 2

Effect of pH on α -amylase adsorption (Embedded Cu²⁺-APPa: 20 mg, α -amylase concentration: 1 mg/mL, Flow rate: 1 mL/min, T: 25 °C) (a); Effect of concentration on α -amylase adsorption (pH: 4.0, Embedded Cu²⁺-APPaC: 20 mg; Flow rate: 1 mL/min; T: 25 °C) (b)

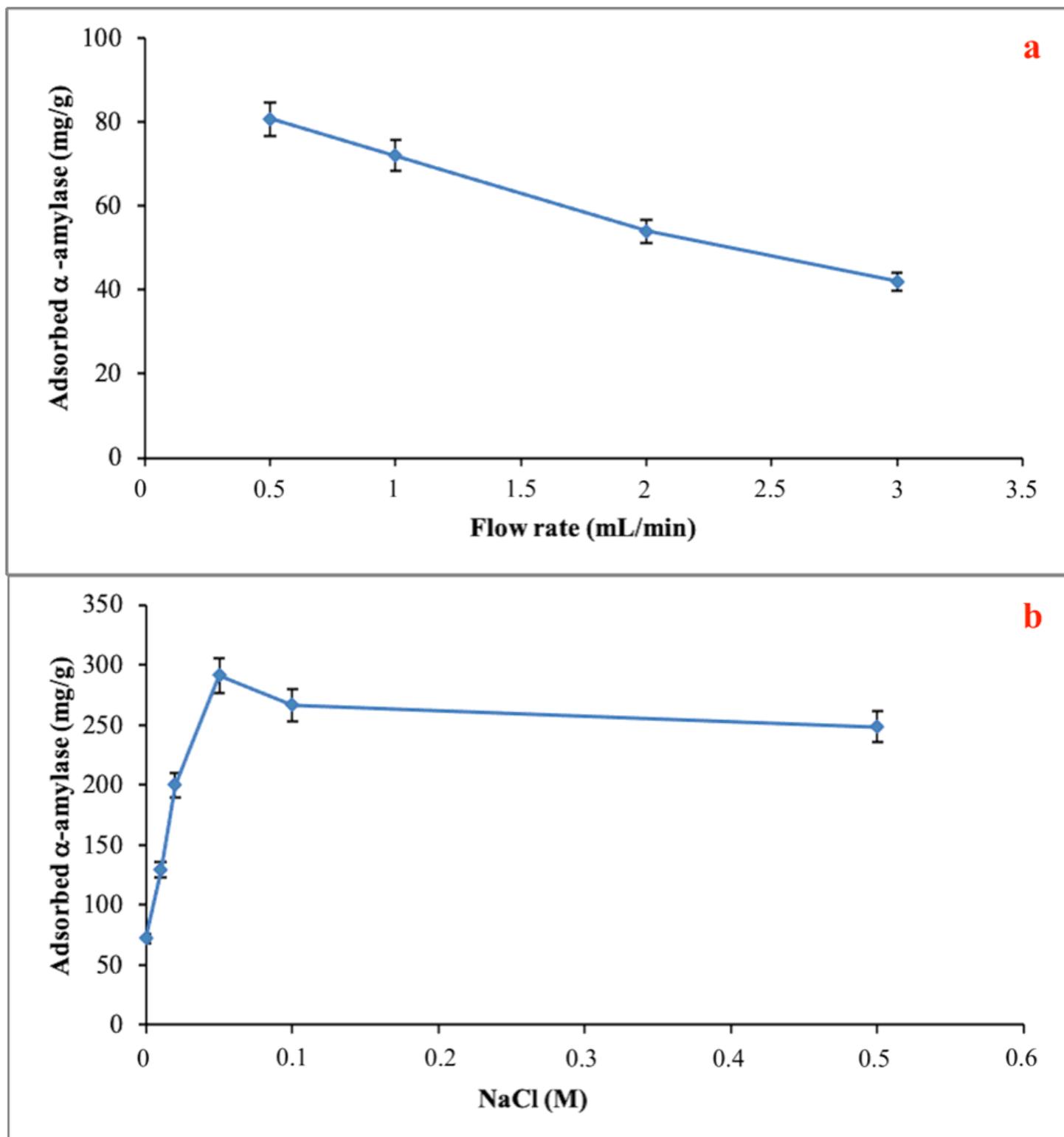


Figure 3

Effect of flow rate on α -amylase adsorption (pH 4.0, Concentration of α -amylase: 1 mg/mL, Embedded Cu^{2+} -APPa: 20 mg, T: 25 °C) (a); Effect of ionic strength on α -amylase adsorption (pH 4.0, Concentration of α -amylase: 1 mg/mL, Embedded Cu^{2+} -APPa: 20 mg, Flow rate: 1 mL/min; T: 25 °C) (b)

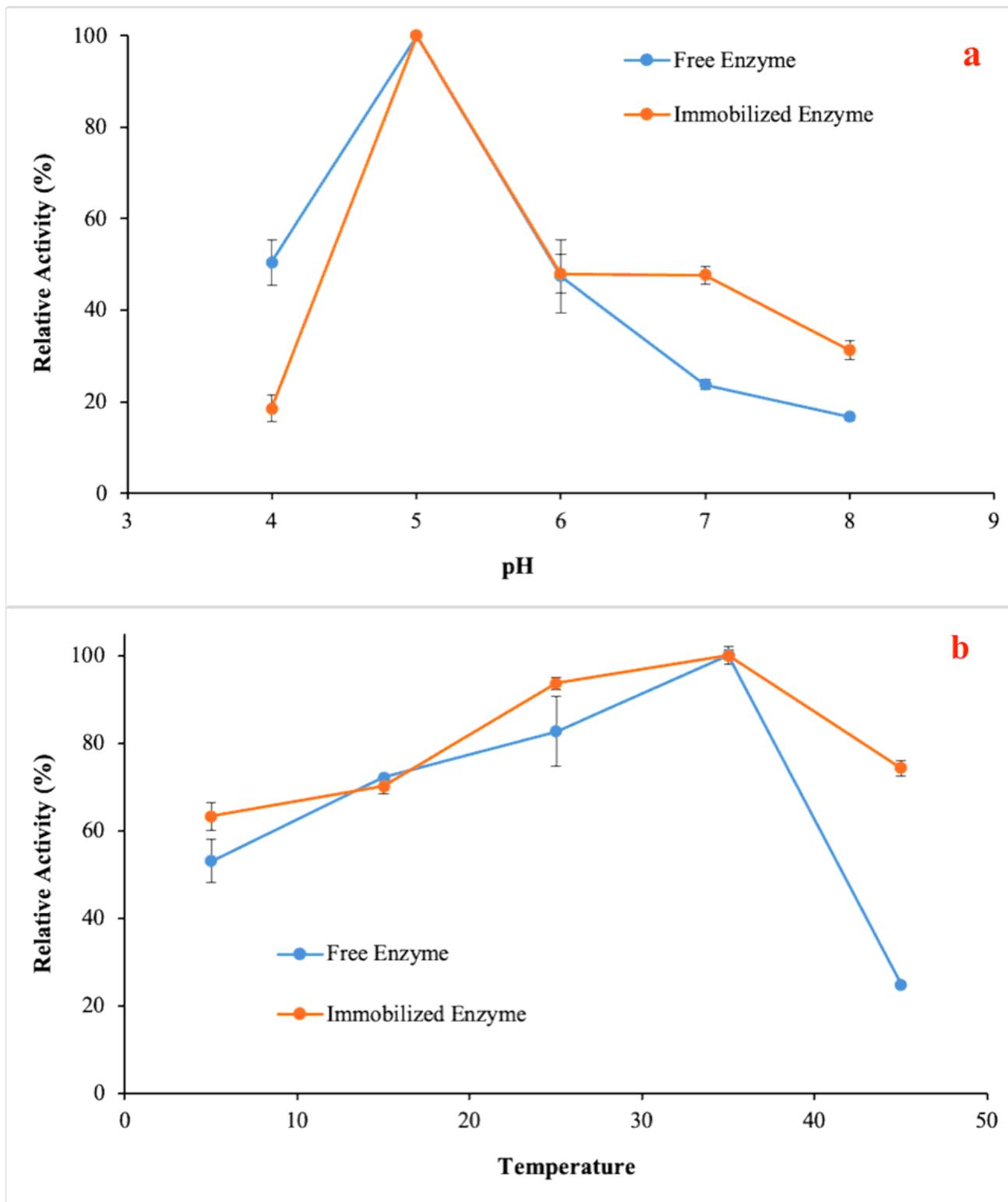


Figure 4

Effect of pH on the free and immobilized amylase activities (Concentration of α -amylase: 1 mg/mL, Embedded Cu^{2+} -APPa: 20 mg, T: 25 °C) (a); Effect of temperature on the free and immobilized amylase activities (Concentration of α -amylase: 1 mg/mL, Embedded Cu^{2+} -APPa: 20 mg; pH:5.0) (b)

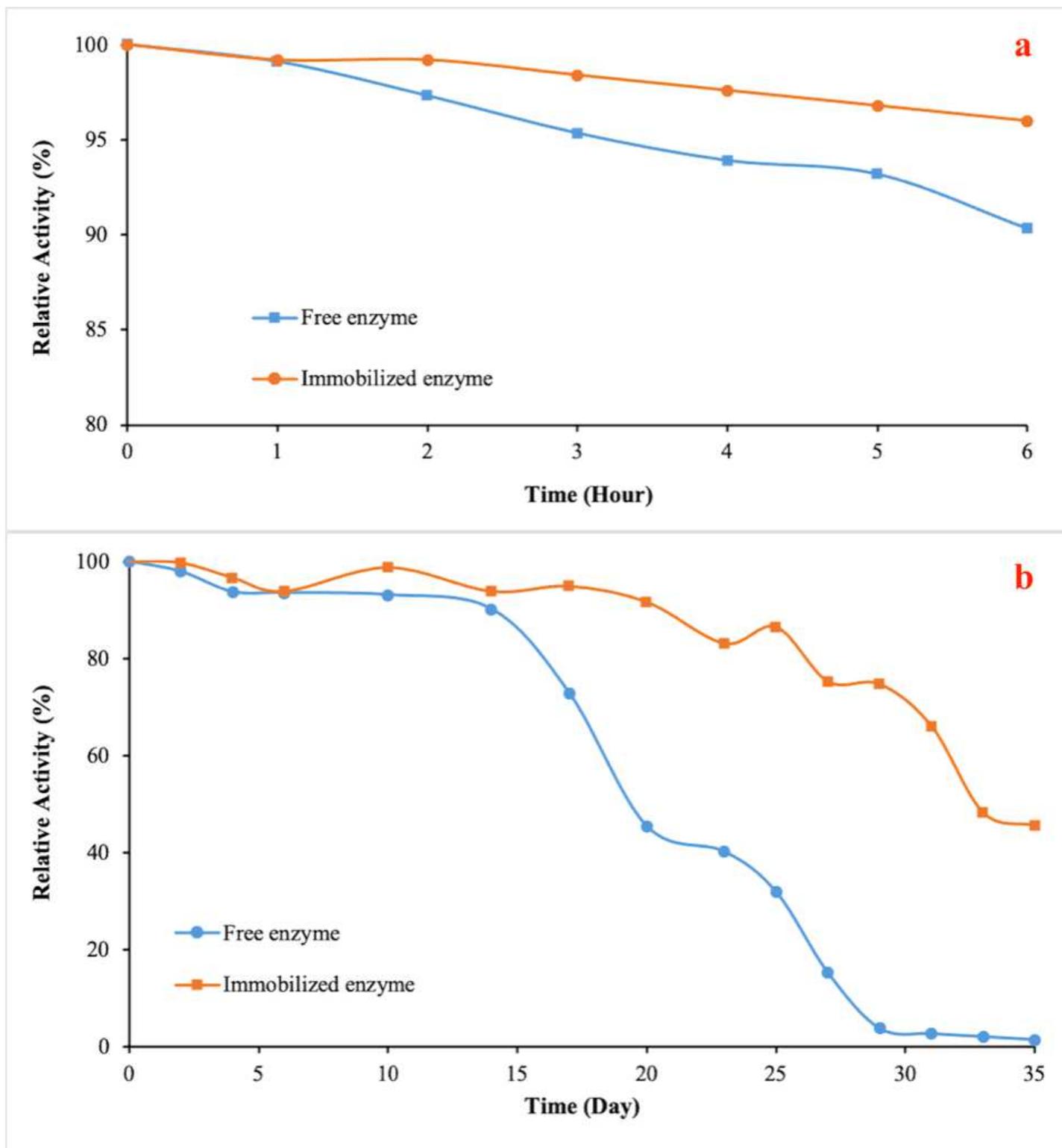


Figure 5

Thermostability of the free and immobilized α -amylase (Concentration of α -amylase: 1 mg/mL, Embedded Cu^{2+} -APPa: 20 mg, pH:5.0) (a); Storage stabilities of the free and immobilized α -amylase (Concentration of α -amylase: 1 mg/mL, Embedded Cu^{2+} -APPa: 20 mg, pH:5.0, T: 4 °C) (b)

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