Carbohydrate Microcapsules Tailored and Grafted for Covalent Immobilization of Glucose Isomerase for Pharmaceutical and Food Industries

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Abstract

Carrageenan is one of the most common carbohydrates utilised in the entrapment industry to immobilise cells and enzymes. However, it lacks functionality. Carrageenan has been grafted to produce fructose by covalently immobilising glucose isomerase (GI). Fructose is one of the most widely used sweeteners in beverages, food production, and the pharmaceutical business. Up to 91.1 U g^{-1} gel beads are immobilised by the grafted beads. Immobilized GI has a V_{max} of 13.8 times that of the free enzyme. The immovability and tolerance of the gel beads immobilised with GI over 15 consecutive cycles were demonstrated in a reusability test, with 88 percent of the enzyme's original activity retained, compared to 60 percent by other authors. These findings are encouraging for high-fructose corn syrup producers.

1. Introduction

The immobilisation approach ensures that the enzyme can be used continuously, making the immobilised enzyme more suited and advantageous than the free-enzyme state. This is due to the ease with which the immobilised enzyme may be regenerated, resulting in a low-cost industrial process. Adsorption (Salah, Srimathi, Gulnara, Ikuo, & Bengt, 2008), crosslinking (Elnashar & Hassan, 2014), entrapment (Betancor, Luckarift, Seo, & Brand, 2008; M. E. Hassan, Tamer, & Omer, 2016), or a combination of these approaches (D'Souza, 1999) are the most often used enzyme immobilization techniques.

In the future, according to Street, GI will be the most significant industrial enzyme (Street, 1977). For example, GI transforms glucose to fructose, which is utilised as a sucrose alternative in the pharmaceutical industry (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2012). Because GI has a financial interest in enzyme immobilisation, a variety of methods are applied, including glutaraldehyde crosslinking (Tyagi, Batra, & Gupta, 1999), entrapment in polyacrylamide (Yuan, Luan, Rana, Hassan, & Dou, 2016), and adsorption on DEAE-Cellulose (Chen & Anderson, 1979). Diethyleaminoethyl cellulose (DEAE-C) (Gul, Rahman, & Hasnain, 2009) and alginate beads have been used to immobilise the gastrointestinal enzyme (Bao, Ma, & Li, 2011).

κ-Carrageenan is one of the most common carriers utilised in the entrapment method of immobilising cells and enzymes (Belyaeva, Della, & Poncelet, 2004). Carrageenans are generally utilised for enzyme immobilisation by the creation of non-covalent bonds (entrapment/encapsulation) due to their lack of functions, according to Elnashar et al., 2020. Unfortunately, propagation of the biocatalyst from the carrier is a common feature of enzyme entrapment in a hydrogel, especially for enzymes with molecular weights less than 300 KDa. Many researchers have worked in this field, and some fascinating results have been obtained (Daniel, Elnashar, & Awad, 2010; Elnashar, Daniel, & Awad, 2009; Yuan et al., 2016).

Carrageenans have poor thermal stability and mechanical qualities, while being biocompatible and affordable. Some study was done to improve their mechanical and thermal properties, and it was discovered that adding 3,6-anhydro-D-galactose 2-sulphate and gum boosted their mechanical strength

(Wahba & Hassan, 2017). To alleviate the problem of low thermal stability, the carrageenan gel was treated with polyamine compounds to generate a polyelectrolyte complex. -Polyamine groups can improve the heat stability of carrageenan gels (Chao, Haugen, & Royer, 1986). To increase the thermal stability of carrageenan gel, natural polyamine, chitosan, and polyethylenimine were utilised (Elnashar, 2010).

GI (EC 5.3.1.5) catalyses the reversible isomerization of D-glucose to D-fructose, making it one of the most significant enzymes in industry. This conversion is critical, particularly in the production of high-fructose corn syrup (HFCS) (Gill, Manhas, & Singh, 2006). Because fructose is sweeter than all other sugars/carbohydrates, there is a financial incentive to produce it. In addition, because fructose has a higher solubility than sucrose, it is less prone to clump in a variety of foods. Despite the fact that fructose is the sweetest of the naturally occuring caloric sweeteners found in fruits, honey, and some vegetables, it should be ingested in moderation because calories are still present.

Immobilized GI can thus offer numerous benefits in biotechnological and industrial applications. Pure fructose (not HFCS), reusability, ease of product separation from the enzyme, and increased enzyme stability at various temperatures and pH levels without affecting enzyme characteristics are just a few of the benefits. In addition to the kinetic constants of immobilised and free GI, several factors have been investigated. Fourier Transform infrared (FTIR) and scanning electron microscopy are used to monitor the steps involved in preparing the hydrogels and immobilising the enzyme (SEM).

The gastrointestinal enzyme (GI) is a high-cost intracellular enzyme (Tumturk, Altinok, Akosy, & Hasirc, 2008). The catalytic process requires large doses of the enzyme to obtain maximal effectiveness, therefore GI has a high Km value, which makes it more expensive. Furthermore, the free GI's Vmax is relatively low. As a result, it's critical to keep GI immobilised on a cost-effective carrier with a low Km/Vmax ratio. Unfortunately, only a few carriers are regarded cost-effective for industrial usage among the several immobilised GI.

Many researchers and governments have aimed to immobilise GI onto carrageenan in order to generate high fructose syrup with high yields. Carrageenan, on the other hand, lacks functions for covalent interactions, therefore this was restricted to physical interaction. The first is to provide additional amine groups to the carrageenan surface, which enable glutaraldehyde reaction while simultaneously functioning as a spacer arm (ligand) to increase the distance between the enzyme and the support, so reducing steric effects and enhancing enzyme immobilisation yield (Bonazza, Manzo, Dos Santos, & Mammarella, 2018). The second is the development of multi-ionic linkages (a network) between the protonated amino groups (cationic polyethylenimine) and the sulphate groups, which hardens the gel beads (anionic carrageenan). This type of ionic network has been shown to be an alternative to covalently crosslinked hydrogels containing sulphate groups in the gel (Elnashar, Yassin, & Kahil, 2008). Given that glutaraldehyde is one of the most widely used activation materials, it is possible to improve enzyme stability by forming a complex with it. Because glutaraldehyde is one of the most widely used activation materials, it can help to improve enzyme stability by forming strong covalent connections

between the enzyme and the support, as illustrated in scheme (1). (Barbosa et al., 2014; Elnashar, M., 2010; Elnashar, Mostafa, Morsy, & Awad, 2013; Elnashar M. & Yassin, A.M., 2009). A schematic picture depicts the grafted formulation of the carrageenan beads immobilising GI covalently, and the chemical modification is demonstrated using FTIR and SEM techniques (scheme (1)). The enzyme loading capacity, on the other hand, is optimised using a short-chain active ligand (to avoid steric hindrance) and varied enzyme doses. The immobilised and free enzymes' optimal pH and temperature are determined, and the Michaelis constants are investigated using a Hans-Woolf plot. Finally, the immobilized enzyme is checked for its reusability and durability overtime for 15 cycles.

2. Materials And Methods

2.1. Materials

Glutaraldehyde solution (GA) (25%), compound CID: 11966249, κ-carrageenan (M.wt.: 154 000; sulfate ester 25%), CHEBI: 10583, was obtained from Fluka (Switzerland). Glutaraldehyde Pentanedial Glutaral 111-30-8, M.wt.: 100.12. Compound CID: 24083: Cobalt chloride 7646-79-9 MFCD00010938, and polyethyleneimine (PEI) (M.wt.: 423), Cat # 468533, were obtained from Sigma-Aldrich (Germany). Magnesium sulfate (99%), 7487-88-9, was obtained from Chemica Limited in Mumbai, India. Compound CID: 24288 M.wt.: 129.84. Glucose anhydrous 50-99-7, extra pure (M.wt.: 180.16) was obtained from Scharlau. Glucose isomerase (EC 5.3.1.5), compound CID: 107526: was produced in our laboratory from Streptomyces *rochei* and used as crude enzyme. Other chemicals were of Analar or equivalent quality.

List of equipment used:

- Fourier Transform Infrared Spectrophotometer - Shimadzu FTIR -
- 8400 S - made in Japan.
- Scanning electron microscopy (SEM, S-590, HITACHI)
- Magnetic Stirrer model- VELP Scientifica - made in Europe.
- Innotech Encapsulator was purchased from the Innotech Co. for preparation of uniform gel beads on the semi pilot scale – made in Switzerland
- Water bath model- memmert. Type WNB22- made in Germany.
- Automatic Pipette (Gilson – made in France.
- Balance – Precisa – XT 220 A - made in Switzerland.
- pH- meter (Jenway 370).
- Shaker “roller mixer”, Stuart.
- Spectrophotometer model Jasco V-630 - made in Europe.

2.1.1. Experimental techniques

Unless otherwise mentioned, all tests were performed in triplicate, and data are presented as means SD (n = 3) unless otherwise noted. The Student's t-test was used to establish the significance level (p-value) of each concentration effect.

2.2. Methods

2.2.1. Preparation of κ-carrageenan beads

κ- -carrageenan powder (2.5 g) was dissolved in warmed 100 mL distilled water (at 70 ^{o}C), yielding 2.5% (w/v) carrageenan gel beads. The Innotech Encapsulator, model IE-50, was used to manufacture uniform gel beads of 300 μm in size, as illustrated in Fig. 1. The gel bead diameter was in the 300 μm range when using a nozzle. Prior to treatment, the beads were hardened by soaking them in 0.3 M potassium chloride (KCl) for 3 hours.

2.2.2. Activation of gel beads

For 3 hours at room temperature, gel beads of κ-carrageenan were immersed in 4% (v/v) Polyethyleneimine (PEI), pH 9.5. Using distilled water, the aminated beads were rinsed to eliminate any unreacted PEI. The GA solution was then used to react with the aminated beads (Carr-PEI) (2.5% for 3 h). The beads were then thoroughly rinsed in distilled water to remove any unreacted GA. Finally, as illustrated in scheme (1), the activated beads were ready for the next phases of immobilisation (Elnashar, 2005).

2.2.3. Enzyme immobilization

To determine the highest enzyme immobilisation efficiency, one gramme of treated gel beads was soaked in solutions of GI solutions comprising 97-1455 units (U) for one hour at room temperature. The -C = N- bond was formed by the interaction of the amino group (-NH_2) in the enzyme with the free -C = O group on glutaraldehyde (scheme (1)). (Elnashar et al., 2014).

2.2.4. Immobilization Efficiency (I.E.) using our locally prepared GI

One gramme of gel beads was soaked for one hour at room temperature in 15 mL GI solutions of 97-1455 U. (RT). From Eq. 1, the immobilisation efficiency (I.E.) was obtained as follows:

$$\mathrm{I.E.} = \frac{\mathrm{U/gGelbeads}}{\mathrm{GILoadingSolution,U}} * 100 \quad \text{Eq. 1}$$

2.2.5. Determination of GI activity

The GI activity was measured according to Dische and Borenfreund's 1951 publication (Dische & Borenfreund, 1951). A volume of 0.2 mL enzyme solution was added to a substrate solution consisting of

0.5 mL sodium phosphate buffer (0.2 M and pH 7.0), 0.2 mL D-glucose (1M), 0.1 mL $MgSO_4.7H_2O$ (0.1 M), 0.1 mL $CoCl_2.6H_2O$ (0.01M), and 2 mL dH_2O. For 1 hour, the reaction mixture was incubated at 70°C (Thi Nguyen & Tran, 2018). After stopping the enzymatic reaction with 2 mL of 0.5 M perchloric acid, the product was analysed under assay conditions. The amount of GI enzyme required to produce 1 unit of GI activity was defined as the amount of GI enzyme needed for the production of 1 µmol of fructose under assay conditions.

2.2.6. pH profile

The effect of varying pHs on free and immobilised enzyme activity was investigated. Five units of immobilised and free GI were incubated for one hour at 70°C in a 10 mL 1 M glucose solution at pH 5–9. (Torres & Batista-Viera, 2017). The activity at each pH has been given as a percentage of the activity at the optimal pH, which is 100% of the activity.

2.2.7. Temperature profile

The effect of temperature on the catalytic activity of immobilised and free GI was investigated by incubating 5 U of both free and immobilised GI in 10 mL of 1 M glucose solution at pH 7 for 1 hour at various temperatures ranging from 40 to 90^{o}C. (Santos et al., 2015; Seyhan & Dilek, 2008). The ideal temperature was discovered, and enzymatic activity at this temperature is expressed as 100% activity, with activity at other temperatures expressed as a percentage of that activity.

2.2.8. K*m* and V*max* of immobilized and free GI

K*m* and V*max* were used to determine the affinity of the immobilised and free enzymes for their substrates. 5U of GI was incubated for 1 hour at 70^{o}C and pH 7 in various concentrations of glucose solutions ranging from 200–2000 mM. The reaction conditions were inspired by Seyhan ST and Dilek A (2008), who employed glucose concentrations ranging from 100 to 1500 mM (Galvão et al., 2008).

2.2.9. Operational stability

The operational stability of immobilised GI was studied. The GI was used for 15 cycles of 60 minutes each to convert glucose to fructose, which was created from prepared whey using β-galactosidase. One-gram beads carrying 8 units of immobilised GI were mixed with 10 mL of β-galactosidase-pretreated whey. The reaction was kept at 70°C for 60 minutes. The amount of fructose was evaluated after the immobilised GI was withdrawn from the reaction mixture. The beads were thoroughly rinsed in buffer solution before being placed in a new substrate solution. This method was done numerous times, with the first activity being 100% and the subsequent activities being expressed as a percentage of the first.

2.2.10. Fourier Transform infrared (FTIR)

At room temperature, the infrared spectra of all formulations in scheme (1) were recorded using an FT-IR (Shimadzu FTIR – 8400 S, Japan) from 4000 to 400 cm^{-1}. FTIR spectra of κ-carrageenan (A), aminated κ-carrageenan (B), activated κ-carrageenan (C), and enzyme immobilised on κ-carrageenan (D) were obtained (D).

2.2.11. Scanning Electron Microscope (SEM)

Four separate sets of beads (κ-carrageenan (A), aminated κ-carrageenan (B), activated κ-carrageenan (C), and enzyme immobilised on κ-carrageenan (D)) were produced and activated as described in the preceding section for comparison reasons. After that, the activated gel beads were either lyophilized immediately or soaked for 18 hours in 0.05 M citrate–phosphate buffer. Scanning Electron Microscopy (SEM; S-590, Hitachi) was used to evaluate the surface morphology of the lyophilized beads in order to investigate the porosity of the gel beads and prove that changes happened on the surface after each reaction.

3. Results And Discussion

The Innotech Encapsulator was utilised to manufacture homogenous gel beads of κ-carrageenan with a size of 300 m, as illustrated in Fig. 1. Figure 2 shows that utilising 1445 U enzyme solutions (crude enzyme, molecular weight ranges from 52,000 to 191,000 according to Chen, 1980) with a 6.3 percent immobilisation effectiveness, a maximum enzyme loading capacity of 91.1 U g-1 gel beads was achieved. However, we chose not to utilise the maximum loaded enzyme in future experiments since it had a low I.E., and it would be more cost-effective to use formulations like those that produced 72 U g-1 gel beads with an I.E. of 37.3 percent. This formula does not indicate the greatest I.E., but it does show the most likely economic value.

To achieve the maximum enzyme loading capacity, we took into consideration several factors such as the ligand’s chain length (Elnashar, M. M., & Hassan, M. E., 2014), as the long chain ligand would enable multilayer/multipoint attachment of the immobilized enzyme. Such steric hindrance might result in a loss of the enzyme’s activity due to a change in its 3D structure.

3.1. Determination of optimum pH profile

Because the interaction of protein groups with the support is affected by the pH, changing the immobilisation pH can change the orientation of enzyme molecules on the support. The last nucleophile blocking of the support allowed for the elimination of the support's chemical reactivity, preventing unwanted enzyme-support covalent connections and serving as a helpful reaction end point (dos Santos et al., 2015). During its isoelectric point, the enzyme does not work well. Because it denatures near this value, the GI isoelectric point is 3. GI is most stable and crystalline in the pH range of 6 to 8, and denatures fast below pH 5 (Carrell et al., 1989).

As shown in Fig. 3, the optimum pHs for the free and immobilized GI were 6.5-7 and 6-7.5, respectively. Statistical analysis (one paired-ample t-test) showed a significant differentiation between the mean results of free and immobilized formulae of enzyme, with P*= 0.052 ($P \leq 0.05$ is significant). The data showed that the immobilization process has increased the enzyme stability (wider pH) compared to the free enzyme. This could be attributed to the fixed configuration of the immobilized GI inside or on the surface of the beads, which increases the stability and tolerability of the enzyme towards the surrounding

pH (Wang et al., 2007). Our results reveal a better enzyme stability when compared to the results obtained by other authors; for example, Arun and Srevastava (2016) found it at pH 6.5 (Arun & Srivastava, 2016), while Hayrettin et al. (2008) and Kamal et al. (2014) found it at pH 7.5 (Hayrettin, Gokhan, Haydar, Serpil, & Nesrin, 2008; Kamal et al., 2014), and Pawar and Deshmukh found it at pH 7.5 (Pawar & Deshmukh, 1994).

3.2. Determination of optimum temperature profile

The thermal stability of covalently immobilized GI onto chemically activated κ-carrageenan beads is one of the most important application criteria for industrial applications (Manoel, dos Santos, Freire, Rueda, & Fernandez-Lafuente, 2015; Palomo et al., 2002). The optimum temperatures for the free and immobilized enzyme were 70 & 65–75 ^{o}C respectively, as shown in Fig. 4. Statistical analysis (one paired -sample t-test) showed a significant differentiation between the mean results of free and immobilized formulae of enzyme, with P*= 0.0380 ($P \leq 0.05$ is significant). The results show that the immobilization process made the enzyme more stable at a wider temperature range than it did the free enzyme. Tor et al. (1989) supports this finding, as he suggested that the immobilization process forms a cage surrounding the enzyme. That cage protects/isolates the immobilized enzyme from the bulk temperature and reduces the temperature of the environment surrounding the enzyme (Tor et al., 1989). High temperature is also favored, to avoid microbial contamination. Our results were comparable to those found in literature, where the optimum temperatures for the immobilized enzyme were 70^{O}C (Buchholz, 1992); 60°C (Fagir & Abu-reesh, 1998; Sorenseon & Emborg, 1989), 65°C (Kamal et al., 2014) and 75°C (Pawar & Deshmukh, 1994). The optimum temperature was also found to be 70°C for the free enzyme, and 85°C for the immobilized enzyme (Ge et al., 1998).

3.3. K*m* and V*max* of free and immobilized GI

The K*m* and Vm*ax* of the immobilized and free GI were calculated by using the Hanes-Woolf plot method as in Fig. 5. Table 1 shows the calculated values. The k*m* for the immobilized enzyme was 492 mM which was higher than that of the free one, 29.3 mM. This indicates that the immobilized enzyme needs more substrate concentrations than does the free one (Hassan, Ran, Yuan, Xiaoning, & Duo, 2019). The increase in K*m* value might be because of diffusional limitations, steric effects and changes in enzyme structure which influence the ability of the substrate to react with the enzyme active sites (Awad, Abd El Aty, Shehata, Hassan, & Elnashar, 2016). The results of the free K*m* is in agreement with published data by Tumturk et al. (2008), as he reported that the K*m* value of free GI was 17.9 mM (Tumturk et al., 2008). The maximum reaction velocity (V*max*) value for the immobilized enzyme, 1117 μmol min^{-1}, was higher than the V*max* of the free enzyme, 81 μmol min^{-1}. The increase of the enzyme's V*max* after immobilization is highly favored in industry, as more products are produced per min compared to the free enzyme. This could be explained as follows: for the enzyme to be efficient, the loss of entropy, coming from the binding between enzyme and substrate to form the enzyme–substrate complex, must be paid by the released binding energy from the favoring interaction between substrate and enzyme (Seyhan & Dilek, 2008). Although the K*m* has increased after the immobilization process, however, the V*max* of the immobilized enzyme has increased too. Using the same amount of free and immobilized enzyme, by

comparing the ratios of the free enzyme's V*max* (81 µmol min^{-1}) to its K*m* (29.3 mM), with that of the immobilized one (K*m*: 492 mM and V*max*: 1117 µmol min^{-1}), the result is 2.76 and 2.27, respectively. The catalytic efficiency (V*max*/K*m*) of the immobilized enzyme was 82.2% of that of the free one, and this could be attributed to product inhibition or slight enzyme mis-conformation after immobilization (Ahmed, Saleh, Abdel-Hameed, & Fayad, 2019). Overall, the result is positive, as the immobilization process has many benefits including operational stability that the free enzyme doesn't afford.

Table 1
Kinetic constants of free and immobilized GI.

GI Form	Kinetic Constants	
	Km (mM)	Vmax (µmol min^{-1})
Free	29.3	81
Immobilized	492	1117

3.4. Operational stability

The most important elements of the industrial applicability of immobilised enzymes are their reuse, and the simplicity of separation. Immobilized enzymes may be more beneficial than free enzymes if their stability is improved. The immobilised GI was employed 15 times for 60 minutes per cycle, as shown in Fig. 6, and the residual activity was roughly 88 percent of the starting activity (Awad et al., 2016). After being encapsulated in poly (acrylic acid-co-2- acrylamido 2-methyl propane sulfonic acid) P (AA-co-AMPS), it preserved 81% of its initial activity after being utilised 15 times (Kamal et al., 2014). When adsorbed on Indion resin, however, it showed inferior stability with repeated use, losing around 40% of its initial activity after five cycles and 61% after the seventh cycle.

3.5. Elucidation of the modified gel beads

3.5.1. Fourier Transform infrared spectroscopy

Figure 7 shows the FT-IR analysis of gel beads at each step of the immobilisation procedure in the range of 400 to 4000 cm^{-1}. Hassan et al., 2019, used the snailase enzyme to create the illustration. The same procedure used to activate k-carrageenan with glutaraldehyde was employed to immobilise GI, as shown in Fig. 7. The activated carrier with free aldehyde groups interacts to the enzyme via its amino groups, which are plentiful in all enzymes, therefore changing the enzyme should not make a difference. The characteristic peaks seen in each formula are depicted in this diagram. The spectra of κ-carrageenan gel beads are shown in curve "A," while curve "B" reveals a new broad peak at 3400 cm^{-1}, indicating the

existence of κ-carrageenan gel beads. This signifies that the amine group is added to the gel beads. The spectra indicate two new peaks after activation with glutaraldehyde, one referring to a free aldehyde end group (C = O) at 1730 cm^{-1} and the other corresponding to an N = C group that has formed between the amine group on the surface of the beads and the aldehyde group found in glutaraldehyde (curve "C"). Curve "D" has a larger peak at 3450 cm^{-1}, showing that the enzyme's amine groups generate a rise in amine group concentrations. We can deduct from the foregoing that the entire immobilisation process was successful. This result was in line with prior research findings (Karam, Hassan, Moharam, & Kansoh, 2018).

3.5.2. Scanning Electron Microscope (SEM)

Figure 8 displays the SEM result for κ-carrageenan, aminated κ-carrageenan, activated κ-carrageenan and immobilized GI onto activated κ-carrageenan (Fig. 8A-8D, respectively). The Figures show the changes that happened on the surface in each step after each treatment. For example, in Fig. 8A (untreated carrageenan), the surface is rough (Whaba, Marwa & Soliman, Tarek, 2018). In Fig. 8B, there is the appearance of many small beads which are attributed to the amination of carrageenan with PEI. In Fig. 8C, the surface is starting to get smoother with a few pebble-structures on its surface which are attributed to the formation of the C = N and free aldehyde end groups (C = O) on its surface as proven in FTIR (Fig. 7). Finally, in Fig. 8D, the surface is getting almost smoother. And that could be attributed to the immobilization of GI on its surface. From the Figures, we can also see the difference in pore size after each step, and this is consistent with other researchers' findings.

4. Conclusions

To create fructose from glucose, the GI enzyme was covalently attached onto -carrageenan gel beads. The pharmaceutical and food industries benefit greatly from the industrial production of fructose because fructose serves as a cryprotectant, which is used to make medicines more pleasant, and promotes hydrophobic active-ingredient solubility. The optimum pH and temperature of the immobilised GI have been shifted to a wider range than the free one, according to the results. This improves the enzyme's stability and tolerance to changes in pH and temperature, as well as preventing microbial contamination. Furthermore, after 15 reuses (each cycle lasting 60 minutes), immobilised GI retains 88% of its action, which is beneficial on an industrial level. These findings support the economic and biotechnological advantages of using GI immobilisation to manufacture pure fructose rather than HFCS (which contains glucose). As a result, the findings of this study are regarded as a good model for producing pure fructose from natural and cost-effective sources. The authors suggest that this method be scaled up on a semi-pilot size in the future. This is not a simple process because it will require the use of a bioreactor and changes to the reaction conditions, such as quantity, thermodynamics, and heat transport. As a result, including a chemical engineer is highly suggested.

Declarations

Conflicts of interest

There is no conflict of interest.

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References

1. Ahmed, S. A., Saleh, S. A. A., Abdel-Hameed, S. A. M., & Fayad, A. M. (2019). Catalytic, kinetic and thermodynamic properties of free and immobilized caseinase on mica glass-ceramics. Heliyon (Vol. 5, p. e01674). https://doi.org/10.1016/j.heliyon.2019.e01674
2. Arun, G., & Srivastava, S. K. (2016). Study of cross linked enzyme aggregate of glucose isomerase of Streptomyces thermonitrificans immoblised on magnetic particle. J Biochem Tech., 7, 1102-1106.
3. Awad, G. E. A., Abd El Aty, A. A., Shehata, A. N., Hassan, M. E., & Elnashar, M. M. (2016). Covalent immobilization of microbial naringinase using novel thermally stable biopolymer for hydrolysis of naringin. 3 Biotech, 6(1), 14. https://doi.org/10.1007/s13205-015-0338-x
4. Bao, Y., Ma, J., & Li, N. (2011). Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly(AA-co-AM-co-AMPS)/ MMT superabsorbent hydrogel. Carbohydr. Polym., 84, 76-82.

https://doi.org/10.1016/j.carbpol.2010.10.061.

5. Barbosa, O., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Rodrigues, R. C., & Fernandez-Lafuente, R. (2014). Glutaraldehyde in bio-catalysts design: a useful crosslinker and a versatile tool in enzyme immobilization. RSC Adv, 4, 1583-1600. https://doi.org/10.1039/C3RA45991H
6. Barclay, T. G., Ginic-Markovic, M., Cooper, P. D., & Petrovsky, N. (2012). The chemistry and sources of fructose and their effect on functionality and health implications. J Excipients and Food Chemicals, 3, 67-82. https://ojs.abo.fi/ojs/index.php/jefc/article/view/145
7. Bazaraa, W. A., & Hamdy, M. K. (1989). Fructose production by immobilizedArthrobacter cells. Journal of Industrial Microbiology, 4(4), 267-274. https://doi.org/10.1007/BF01577349
8. Belyaeva, E., Della, V. D., & Poncelet, D. (2004). Immobilization of α-chymotrypsin in k-carrageenan beads prepared with the static mixer. Enzyme Microb. Technol., 34, 108-113. https://doi.org/10.1016/j.enzmictec.2003.07.009
9. Betancor, L., Luckarift, R., Seo, H., & Brand, O. (2008). Three-dimensional immobilization of β-galactosidase on a silicon surface. Biotechnol. Bioeng., 99, 261- 267. https://doi.org/10.1002/bit.21570
10. Bonazza, H. L., Manzo, R. M., Dos Santos, J. C. S., & Mammarella, E. J. (2018). Operational and thermal stability analysis of thermomyces lanuginosus lipase covalently immobilized onto modified chitosan supports. Appl Biochem Biotechnol, 184(1), 182-196. https://doi.org/10.1007/s12010-017-2546-9
11. Buchholz, K. (1992). Immobilized enzymes. kinetics, efficiency, and applications. Inter. J. Chem. Eng., 32, 1-13.
12. Carrell, H. L., Glusker, J. P., Burger, V., Manfre, F., Tritsch, D., & Biellmann, J. F. (1989). X-ray analysis of D-xylose isomerase at 1.9 A: native enzyme in complex with substrate and with a mechanism-designed inactivator. Proc Natl Acad Sci USA, 86(12), 4440-4444. DOI: 10.1073/pnas.86.12.4440
13. Chao, K. C., Haugen, M. M., & Royer, G. P. (1986). Stabilization of kappa-carrageenan gel with polymeric amines: Use of immobilized cells as biocatalysts at elevated temperatures. Biotechnol Bioeng, 28(9), 1289-1293. DOI: 10.1002/bit.260280902.
14. Chen, W. P. 1980. Glucose isomerase. Process Biochem. 15(August/September):36–41. Chen, W. P., & Anderson, A. W. (1979). Purification, immobilization, and some Properties of glucose isomerase from streptomyces flavogriseus. Appl Environ Microbiol, 38(6), 1111-1119. https://doi.org/10.1128/AEM.38.6.1111-1119.1979
15. D'Souza, S. F. (1999). Immobilized enzymes in bioprocess. Current Science, 77, 69.
16. Daniel, E. N., Elnashar, M. M., & Awad, G. E. (2010). Immobilized Inulinase on Grafted Alginate Beads Prepared by the One-Step and the Two-Steps Methods. Ind. Eng. Chem. Res., 49, 3120–3125. https://doi.org/10.1021/ie100011z
17. Deshmukh, S. S., Dutta, C. M., & Shankar, V. (1993). Preparation and properties of glucose isomerase immobilized on Indion 48-R. Appl. Biochem. Biotechnol., 42, 95-104. https://doi.org/10.1007/BF02788045

18. Dische, Z., & Borenfreund, E. (1951). A new spectrophotometric method for the detection and determination of keto sugars and trioses. J Biol Chem, 192(2), 583-587. PMID: 14907652
19. dos Santos, J. C. S., Rueda, N., Barbosa, O., Millán-Linares, M., Pedroche, J., del Mar Yuste, M., . . . Fernandez-Lafuente, R. (2015). Bovine trypsin immobilization on agarose activated with divinylsulfone: Improved activity and stability via multipoint covalent attachment. Journal of Molecular Catalysis B: Enzymatic, 117, 38-44. https://doi.org/10.1016/j.molcatb.2015.04.008
20. Elnashar, M. M. (2005). Carrageenan treated with synthetic polymer as a carrier for biotechnological applications. In E. P. Office (Ed.). Egypt Patent EG20050000443. 11 Oct 2005.
21. Elnashar, M. M. (2010). Review article: Immobilized Molecules Using Biomaterials and Nanobiotechnology. Journal of Biomaterials and Nanobiotechnology, 1, 61-76. DOI: 10.4236/jbnb.2010.11008
22. Elnashar, M. M., Awad, G. E., Hassan, M. E., Mohy Eldin, M. S., Haroun, B. M., & El-Diwany, A. I. (2014). Optimal immobilization of beta-galactosidase onto kappa-carrageenan gel beads using response surface methodology and its applications. Scientific World Journal, 2014, 571682. https://doi.org/10.1155/2014/571682
23. Elnashar, M. M., Daniel, E. N., & Awad, G. E. (2009). Novel Carrier of Grafted Alginate for Covalent Immobilization of Inulinase. Ind. Eng. Chem. Res., 48, 9781–9785. https://doi.org/10.1021/ie9011276
24. Elnashar, M. M., & Hassan, M. E. (2014). Novel epoxy activated hydrogels for solving lactose intolerance. Biomed Res Int, 2014, 817985. https://doi.org/10.1155/2014/817985
25. Elnashar, M. M., Hassan, M. E., & Awad, G. E. A. (2013). Grafted carrageenan gel disks and beads with polyethylenimine and glutaraldehyde for covalent immobilization of penicillin G acylase J. Colloid Sci. Biotechnol., 2, 1–7. DOI.10.1166/jcsb.2013.1029
26. Elnashar, M. M., Mostafa, H., Morsy, N. A., & Awad, G. E. A. (2013). Biocatalysts: Isolation, Identification and Immobilization of Thermally Stable Lipase onto Novel Supports. Industrial & Engineering Chemistry Research, 52, 14760–14767. https://doi.org/10.1021/ie402960d
27. Elnashar, M. M., & Yassin, A. M. (2009). Covalent immobilization of β-galactosidase on carrageenan coated chitosan. Journal of Applied Polymer Sciences, 114, 17-24. https://doi.org/10.1002/app.30535
28. Elnashar, M. M., Yassin, A. M., & Kahil, T. (2008). Novel Thermally and Mechanically Stable Hydrogel for Enzyme Immobilization of Penicillin G Acylase via Covalent Technique. Journal of Applied Polymer Sciences, 109 4105-4111. https://doi.org/10.1002/app.28379
29. Elnashar, M. M., & Yassin, M. A. (2009). Lactose hydrolysis by beta-galactosidase covalently immobilized to thermally stable biopolymers. Appl Biochem Biotechnol, 159(2), 426-437. DOI: 10.1007/s12010-008-8453-3
30. Elnashar, M. M. M. (2010). Review article: Immobilized Molecules Using Biomaterials and Nanobiotechnology. Biomaterial Nanobiotechnology, 1(61). DOI:10.4236/jbnb.2010.11008

31. Fagir, N. M., & Abu-reesh, I. M. (1998). Optimum temperature operation mode for glucose isomerase reactor operating at glucose conversion. Bioprocess. Eng., 19, 11–17. https://doi.org/10.1007/s004490050476
32. Fernandez-Lopez, L., Pedrero, S. G., Lopez-Carrobles, N., Gorines, B. C., Virgen-Ortíz, J. J., & Fernandez-Lafuente, R. (2017). Effect of protein load on stability of immobilized enzymes. Enzyme and Microbial Technology, 98, 18-25. DOI: 10.1016/j.enzmictec.2016.12.002
33. Galvão WS, P. B., Golçalves LRB, de Mattos MC, Fonseca TS, Regis T, Fechine PBA (2018). Novel nanohybrid biocatalyst: application in the kinetic resolution of secondary alcohols. . J. Mater. Sci., 53, 14121-14137. DOI: 10.1007/s10853-018-2641-5
34. Ge, Y. B., Zhou, H., Kong, W., Tong, Y., Wang, S. Y., & Li, W. (1998). Immobilization of glucose isomerase and its application in continuous production of high fructose syrup. Appl. Biochem. Biotechnol., 69, 203– 215. https://doi.org/10.1007/BF02788814
35. Gill, P. K., Manhas, R. K., & Singh, P. (2006). Hydrolysis of inulin by immobilized thermostable extracellular exoinulinase from Aspergillus fumigatus. Journal of Food Engineering, 76(3), 369-375. DOI: 10.1016/j.jfoodeng.2005.05.052
36. Gul, A., Rahman, M. A., & Hasnain, S. N. (2009). Role of fructose concentration on cataractogenesis in senile diabetic and nondiabetic patients. Graefe's Archive for Clinical and Experimental Ophthalmology, 247 809 - 814. DOI: 10.1007/s00417-008-1027-9
37. Hassan, M., Ran, X., Yuan, Y., Xiaoning, L., & Duo, D. (2019). Biotransformation of ginsenoside using covalently immobilized snailase enzyme onto activated carrageenan gel beads. Bull. Mater. Sci., 42, 29. https://doi.org/10.1007/s12034-019-1730-7
38. Hassan, M. E., Tamer, T. M., & Omer, A. M. (2016). Methods of enzyme immobilization Inter J Curr Pharma Rev and Res., 7 385-392. Corpus ID: 92984962
39. Hassan, M. E., Yang, Q., & Xiao, Z. (2019). Covalent immobilization of glucoamylase enzyme onto chemically activated surface of κ-carrageenan. Bulletin of the National Research Centre, 43, 102-112. https://doi.org/10.1186/s42269-019-0148-0
40. Hayrettin, T., Gokhan, D., Haydar, A., Serpil, A., & Nesrin, H. (2008). Immobilization of glucose isomerase in surface-modified alginate gel beads. . J Food Biochem, 32, 234–246. https://doi.org/10.1111/j.1745-4514.2008.00171.x
41. Kamal, H., EL-Sayed, A. H., Hayat, M. S., Sawsan, A. A., Lotfy, S., & Randa, D. M. (2014). Immobilization of glucose isomerase onto radiation synthesized P(AA-co-AMPS) hydrogel and its application. J Radia Rese and Appl Sci., 7, 154–162. https://doi.org/10.1016/j.jrras.2014.02.001
42. Karam, E. A., Hassan, M. E., Moharam, M. E., & Kansoh, A. L. (2018). Immobilization of inulinase produced by Rhizopus oligosporus NRRL 2549 for continuous fructose production. J. Mater. Environ. Sci., 9, 2315 - 2321.
43. Manoel, E. A., dos Santos, J. C. S., Freire, D. M. G., Rueda, N., & Fernandez-Lafuente, R. (2015). Immobilization of lipases on hydrophobic supports involves the open form of the enzyme. Enzyme and Microbial Technology, 71, 53-57. DOI: 10.1016/j.enzmictec.2015.02.001

44. Manoel, E. A., Ribeiro, M. F. P., dos Santos, J. C. S., Coelho, M. A. Z., Simas, A. B. C., Fernandez-Lafuente, R., & Freire, D. M. G. (2015). Accurel MP 1000 as a support for the immobilization of lipase from Burkholderia cepacia: Application to the kinetic resolution of myo-inositol derivatives. Process Biochemistry, 50(10), 1557-1564. DOI:10.1016/j.procbio.2015.06.023
45. Palomo, J. M., Muñoz, G., Fernández-Lorente, G., Mateo, C., Fernández-Lafuente, R., & Guisán, J. M. (2002). Interfacial adsorption of lipases on very hydrophobic support (octadecyl–Sepabeads): immobilization, hyperactivation and stabilization of the open form of lipases. Journal of Molecular Catalysis B: Enzymatic, 19-20, 279-286. https://doi.org/10.1016/S1381-1177(02)00178-9
46. Pawar, H. S., & Deshmukh, D. R. (1994). Immobilization of D-xylose (D-glucose) isomerase from a Chainia species. Prep Biochem, 24(2), 143-150. doi:10.1080/10826069408010088.
47. Salah, S., Srimathi, S., Gulnara, S., Ikuo, S., & Bengt, D. (2008). Hydroxyapatite as a novel reversible in situ adsorption matrix for enzyme thermistor-based FIA. Talanta, 77, 490-493. DOI: 10.1016/j.talanta.2008.04.003
48. Santos, J. C. S., Barbosa, O., Ortiz, C., Berenguer-Murcia, A., Rodrigues, R. C., & Fernandez-Lafuente, R. (2015). Importance of the support properties for immobilization or purification of enzymes. Chem. Cat. Chem, 7, 2413-2432. https://doi.org/10.1002/cctc.201500310
49. Seyhan, S. T., & Dilek, A. (2008). Catalytic efficiency of immobilized glucose isomerase in isomerization of glucose to fructose. Food Chemistry, 111, 658–662. DOI: 10.1016/j.foodchem.2008.04.035
50. Sorenseon, J. E., & Emborg, C. (1989). Influence of immobilization technique on the quality of immobilized enzyme products based on a porous SiO2 carrier. Enzyme Microbiol. Technol, 11, 26–28. https://doi.org/10.1016/0141-0229(89)90109-9
51. Street, G. (1977). Handbook of enzyme biotechnology: Edited by Alan Wiseman. Pp. 276. Published by Ellis Horwood Ltd., Chichester, U.K. and distributed by John Wiley and Sons Ltd., 1975, £15.00 or $28.50. Biochemical Education, 5(3), 64-64. https://doi.org/10.1016/0307-4412(77)90054-1
52. Thi Nguyen, H. Y., & Tran, G. B. (2018). Optimization of fermentation conditions and media for production of glucose isomerase from Bacillus megaterium using response surface methodology. Scientifica (Cairo), 2018, 6842843. DOI: 10.1155/2018/6842843
53. Torres, P., & Batista-Viera, F. (2017). Immobilized Trienzymatic System with Enhanced Stabilization for the Biotransformation of Lactose. Molecules, 22(2), 284-298. doi:10.3390/molecules22020284.
54. Tumturk, H., Altinok, H., Akosy, S., & Hasirc, N. (2008). Immobilization of glucose isomerase in surface-modified alginate gel beads. Journal of Food Biochemistry, 32 234–246. https://doi.org/10.1111/j.1745-4514.2008.00171.x
55. Tyagi, R., Batra, R., & Gupta, M. N. (1999). Amorphous enzyme aggregates: Stability toward heat and aqueous-organic cosolvent mixtures. Enzyme Microbiol Technology, 24, 348–354. https://doi.org/10.1016/S0141-0229(98)00131-8
56. Wahba, M. I., & Hassan, M. E. (2017). Agar-carrageenan hydrogel blend as a carrier for the covalent immobilization of β-D-galactosidase. Macromol. Res., 25, 913-923. https://doi.org/10.1007/s13233-

017-5123-8

57. Yuan, Y., Luan, X., Rana, X., Hassan, M. E., & Dou, D. (2016). Covalent immobilization of cellulase in application of biotransformation of ginsenoside Rb1. Journal of Molecular Catalysis B: Enzymatic, 133, S525-S532. https://doi.org/10.1016/j.molcatb.2017.05.004
58. ics. *Heliyon* (Vol. 5, p. e01674).
59. Arun, G., & Srivastava, S. K. (2016). Study of cross linked enzyme aggregate of glucose isomerase of Streptomyces thermonitrificans immoblised on magnetic particle. *J Biochem Tech.,* 7, 1102-1106
60. Awad, G. E. A., Abd El Aty, A. A., Shehata, A. N., Hassan, M. E., & Elnashar, M. M. (2016). Covalent immobilization of microbial naringinase using novel thermally stable biopolymer for hydrolysis of naringin. *3 Biotech,* 6(1), 14.
61. Bao, Y., Ma, J., & Li, N. (2011). Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly(AA-co-AM-co-AMPS)/ MMT superabsorbent hydrogel. *Carbohydr. Polym.,* 84, 76-82.
62. Barbosa, O., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Rodrigues, R. C., & Fernandez-Lafuente, R. (2014). Glutaraldehyde in bio-catalysts design: a useful crosslinker and a versatile tool in enzyme immobilization. *RSC Adv,* 4, 1583-1600.
63. Barclay, T. G., Ginic-Markovic, M., Cooper, P. D., & Petrovsky, N. (2012). The chemistry and sources of fructose and their effect on functionality and health implications. *J Excipients and Food Chemicals,* 3, 67-82.
64. Bazaraa, W. A., & Hamdy, M. K. (1989). Fructose production by immobilizedArthrobacter cells. *Journal of Industrial Microbiology,* 4(4), 267-274.
65. Belyaeva, E., Della, V. D., & Poncelet, D. (2004). Immobilization of α-chymotrypsin in k-carrageenan beads prepared with the static mixer. *Enzyme Microb. Technol.,* 34, 108-113.
66. Betancor, L., Luckarift, R., Seo, H., & Brand, O. (2008). Three-dimensional immobilization of β-galactosidase on a silicon surface. *Biotechnol. Bioeng.,* 99, 261- 267.
67. Bonazza, H. L., Manzo, R. M., Dos Santos, J. C. S., & Mammarella, E. J. (2018). Operational and thermal stability analysis of thermomyces lanuginosus lipase covalently immobilized onto modified chitosan supports. *Appl Biochem Biotechnol,* 184(1), 182-196.
68. Buchholz, K. (1992). Immobilized enzymes. kinetics, efficiency, and applications. *Inter. J. Chem. Eng.,* 32, 1-13.
69. Carrell, H. L., Glusker, J. P., Burger, V., Manfre, F., Tritsch, D., & Biellmann, J. F. (1989). X-ray analysis of D-xylose isomerase at 1.9 A: native enzyme in complex with substrate and with a mechanism-designed inactivator. *Proc Natl Acad Sci USA,* 86(12), 4440-4444.
70. Chao, K. C., Haugen, M. M., & Royer, G. P. (1986). Stabilization of kappa-carrageenan gel with polymeric amines: Use of immobilized cells as biocatalysts at elevated temperatures. *Biotechnol Bioeng,* 28(9), 1289-1293.
71. Chen, W. P., & Anderson, A. W. (1979). Purification, immobilization, and some Properties of glucose isomerase from streptomyces flavogriseus. *Appl Environ Microbiol,* 38(6), 1111-1119.

72. D'Souza, S. F. (1999). Immobilized enzymes in bioprocess. *Current Science,* 77, 69.
73. Daniel, E. N., Elnashar, M. M., & Awad, G. E. (2010). Immobilized Inulinase on Grafted Alginate Beads Prepared by the One-Step and the Two-Steps Methods. *Ind. Eng. Chem. Res.,* 49, 3120–3125.
74. Deshmukh, S. S., Dutta, C. M., & Shankar, V. (1993). Preparation and properties of glucose isomerase immobilized on Indion 48-R. *Appl. Biochem. Biotechnol.,* 42, 95-104.
75. Dische, Z., & Borenfreund, E. (1951). A new spectrophotometric method for the detection and determination of keto sugars and trioses. *J Biol Chem,* 192(2), 583-587.
76. dos Santos, J. C. S., Rueda, N., Barbosa, O., Millán-Linares, M., Pedroche, J., del Mar Yuste, M., . . . Fernandez-Lafuente, R. (2015). Bovine trypsin immobilization on agarose activated with divinylsulfone: Improved activity and stability via multipoint covalent attachment. *Journal of Molecular Catalysis B: Enzymatic,* 117, 38-44.
77. Elnashar, M. M. (2005). Carrageenan treated with synthetic polymer as a carrier for biotechnological applications. In E. P. Office (Ed.). Egypt.
78. Elnashar, M. M. (2010). Review article: Immobilized Molecules Using Biomaterials and Nanobiotechnology. *Journal of Biomaterials and Nanobiotechnology,* 1, 61-76.
79. Elnashar, M. M., Awad, G. E., Hassan, M. E., Mohy Eldin, M. S., Haroun, B. M., & El-Diwany, A. I. (2014). Optimal immobilization of beta-galactosidase onto kappa-carrageenan gel beads using response surface methodology and its applications. *ScientificWorldJournal,* 2014, 571682.
80. Elnashar, M. M., Daniel, E. N., & Awad, G. E. (2009). Novel Carrier of Grafted Alginate for Covalent Immobilization of Inulinase. *Ind. Eng. Chem. Res.,* 48, 9781–9785.
81. Elnashar, M. M., & Hassan, M. E. (2014). Novel epoxy activated hydrogels for solving lactose intolerance. *Biomed Res Int,* 2014, 817985.
82. Elnashar, M. M., Hassan, M. E., & Awad, G. E. A. (2013). Grafted carrageenan gel disks and beads with polyethylenimine and glutaraldehyde for covalent immobilization of penicillin G acylase *J. Colloid Sci. Biotechnol.,* 2, 1–7.
83. 16. Elnashar, M.M. Vaccarezza, M., Al-Salami, H. (2020). Cutting-edge biotechnological advancement in islet delivery using pancreatic and cellular approaches. Future Science OA. https://doi.org/10.2144/fsoa-2020-0105
84. Elnashar, M. M., Mostafa, H., Morsy, N. A., & Awad, G. E. A. (2013). Biocatalysts: Isolation, Identification and Immobilization of Thermally Stable Lipase onto Novel Supports. *Industrial & Engineering Chemistry Research,* 52, 14760–14767.
85. Elnashar, M. M., & Yassin, A. M. (2009). Covalent immobilization of β-galactosidase on carrageenan coated chitosan. *Journal of Applied Polymer Sciences,* 114, 17-24.
86. Elnashar, M. M., Yassin, A. M., & Kahil, T. (2008). Novel Thermally and Mechanically Stable Hydrogel for Enzyme Immobilization of Penicillin G Acylase via Covalent Technique. *Journal of Applied Polymer Sciences,* 109 4105-4111.

87. Elnashar, M. M., & Yassin, M. A. (2009). Lactose hydrolysis by beta-galactosidase covalently immobilized to thermally stable biopolymers. *Appl Biochem Biotechnol,* 159(2), 426-437.
88. Elnashar, M. M. M. (2010). Review article: Immobilized Molecules Using Biomaterials and Nanobiotechnology. *Biomaterial Nanobiotechnology,* 1(61).
89. Fagir, N. M., & Abu-reesh, I. M. (1998). Optimum temperature operation mode for glucose isomerase reactor operating at glucose conversion. *Bioprocess. Eng.,* 19, 11–17.
90. Galvão WS, P. B., Golçalves LRB, de Mattos MC, Fonseca TS, Regis T, Fechine PBA (2018). Novel nanohybrid biocatalyst: application in the kinetic resolution of secondary alcohols. . *J. Mater. Sci.,* 53, 14121-14137.
91. Galvão, W. S., Pinheiro, B. B., Golçalves, L. R. B., de Mattos, M. C., Fonseca, T. S., Regis, T., & Fechine, P. B. A. (2008). Novel nanohybrid biocatalyst: application in the kinetic resolution of secondary alcohols. *J. Mater. Sci.,* 53, 14121-14137.
92. Ge, Y. B., Zhou, H., Kong, W., Tong, Y., Wang, S. Y., & Li, W. (1998). Immobilization of glucose isomerase and its application in continuous production of high fructose syrup. *Appl. Biochem. Biotechnol.,* 69, 203– 215.
93. Gill, P. K., Manhas, R. K., & Singh, P. (2006). Hydrolysis of inulin by immobilized thermostable extracellular exoinulinase from Aspergillus fumigatus. *Journal of Food Engineering,* 76(3), 369-375.
94. Gul, A., Rahman, M. A., & Hasnain, S. N. (2009). Role of fructose concentration on cataractogenesis in senile diabetic and nondiabetic patients. *Graefe's Archive for Clinical and Experimental Ophthalmology,* 247 809 - 814.
95. Hassan, M., Ran, X., Yuan, Y., Xiaoning, L., & Duo, D. (2019). Biotransformation of ginsenoside using covalently immobilized snailase enzyme onto activated carrageenan gel beads. *Bull. Mater. Sci.,* 42, 29.
96. Hassan, M. E., Tamer, T. M., & Omer, A. M. (2016). Methods of enzyme immobilization *Inter J Curr Pharma Rev and Res.,* 7 385-392.
97. Hassan, M. E., Yang, Q., & Xiao, Z. (2019). Covalent immobilization of glucoamylase enzyme onto chemically activated surface of κ-carrageenan. *Bulletin of the National Research Centre,* 43, 102-112.
98. Hayrettin, T., Gokhan, D., Haydar, A., Serpil, A., & Nesrin, H. (2008). Immobilization of glucose isomerase in surface-modified alginate gel beads. . *J Food Biochem,* 32, 234–246.
99. Kamal, H., EL-Sayed, A. H., Hayat, M. S., Sawsan, A. A., Lotfy, S., & Randa, D. M. (2014). Immobilization of glucose isomerase onto radiation synthesized P(AA-co-AMPS) hydrogel and its application. *J Radia Rese and Appl Sci.,* 7, 154–162.
100. Karam, E. A., Hassan, M. E., Moharam, M. E., & Kansoh, A. L. (2018). Immobilization of inulinase produced by Rhizopus oligosporus NRRL 2549 for continuous fructose production. *J. Mater. Environ. Sci.,* 9, 2315 - 2321.
101. Manoel, E. A., dos Santos, J. C. S., Freire, D. M. G., Rueda, N., & Fernandez-Lafuente, R. (2015). Immobilization of lipases on hydrophobic supports involves the open form of the enzyme. *Enzyme and Microbial Technology,* 71, 53-57.

102. Manoel, E. A., Ribeiro, M. F. P., dos Santos, J. C. S., Coelho, M. A. Z., Simas, A. B. C., Fernandez-Lafuente, R., & Freire, D. M. G. (2015). Accurel MP 1000 as a support for the immobilization of lipase from Burkholderia cepacia: Application to the kinetic resolution of myo-inositol derivatives. *Process Biochemistry,* 50(10), 1557-1564.
103. Palomo, J. M., Muñoz, G., Fernández-Lorente, G., Mateo, C., Fernández-Lafuente, R., & Guisán, J. M. (2002). Interfacial adsorption of lipases on very hydrophobic support (octadecyl–Sepabeads): immobilization, hyperactivation and stabilization of the open form of lipases. *Journal of Molecular Catalysis B: Enzymatic,* 19-20, 279-286.
104. Pawar, H. S., & Deshmukh, D. R. (1994). Immobilization of D-xylose (D-glucose) isomerase from a Chainia species. *Prep Biochem,* 24(2), 143-150.
105. Salah, S., Srimathi, S., Gulnara, S., Ikuo, S., & Bengt, D. (2008). Hydroxyapatite as a novel reversible in situ adsorption matrix for enzyme thermistor-based FIA. *Talanta,* 77, 490-493.
106. Santos, J. C. S., Barbosa, O., Ortiz, C., Berenguer-Murcia, A., Rodrigues, R. C., & Fernandez-Lafuente, R. (2015). Importance of the support properties for immobilization or purification of enzymes. *Chem. Cat. Chem,* 7, 2413-2432.
107. Seyhan, S. T., & Dilek, A. (2008). Catalytic efficiency of immobilized glucose isomerase in isomerization of glucose to fructose. *Food Chemistry,* 111, 658–662.
108. Sorenseon, J. E., & Emborg, C. (1989). Influence of immobilization technique on the quality of immobilized enzyme products based on a porous SiO2 carrier. *Enzyme Microbiol. Technol,* 11, 26–28.
109. Street, G. (1977). Handbook of enzyme biotechnology: Edited by Alan Wiseman. Pp. 276. Published by Ellis Horwood Ltd., Chichester, U.K. and distributed by John Wiley and Sons Ltd., 1975, £15.00 or $28.50. *Biochemical Education,* 5(3), 64-64.
110. Thi Nguyen, H. Y., & Tran, G. B. (2018). Optimization of fermentation conditions and media for production of glucose isomerase from Bacillus megaterium using response surface methodology. *Scientifica (Cairo),* 2018, 6842843.
111. Tor, R.; Dror, Y.; Freeman, A (1989). Enzyme stabilization by bilayer “encagement”. *Enzyme Microbial Technology*. 306-312.
112. Torres, P., & Batista-Viera, F. (2017). Immobilized Trienzymatic System with Enhanced Stabilization for the Biotransformation of Lactose. *Molecules,* 22(2), 284-298.
113. Tumturk, H., Altinok, H., Akosy, S., & Hasirc, N. (2008). Immobilization of glucose isomerase in surface-modified alginate gel beads. *Journal of Food Biochemistry,* 32 234–246.
114. Tyagi, R., Batra, R., & Gupta, M. N. (1999). Amorphous enzyme aggregates: Stability toward heat and aqueous-organic cosolvent mixtures. *Enzyme Microbiol Technology,* 24, 348–354.
115. Wahba, M. I., Soliman, T. (2018). Whey protein isolate for the preparation of covalent immobilization beads. Biocatalysis and Agricultural Biotechnology, 14, 328-337./
116. Wahba, M. I., & Hassan, M. E. (2017). Agar-carrageenan hydrogel blend as a carrier for the covalent immobilization of β-D-galactosidase. Macromolecules Research, 25, 913-923.

117. Wang, W., Deng, L., Peng, Z. H., & Xiao, X. (2007). Study of the epoxydized magnetic hydroxyl particles as a carrier for immobilizing penicillin G acylase. Enzyme and Microbial Technology, 40(2), 255-261.
118. Yuan, Y., Luan, X., Rana, X., Hassan, M. E., & Dou, D. (2016). Covalent immobilization of cellulase in application of biotransformation of ginsenoside Rb1. *Journal of Molecular Catalysis B: Enzymatic,* 133, S525-S532.

Scheme

Scheme 1 is available in the Supplementary Files section.

Figures

Figure 1

Encapsulator for making uniform gel beads.

Reprint from Elnashar et al., 2014 (open access).

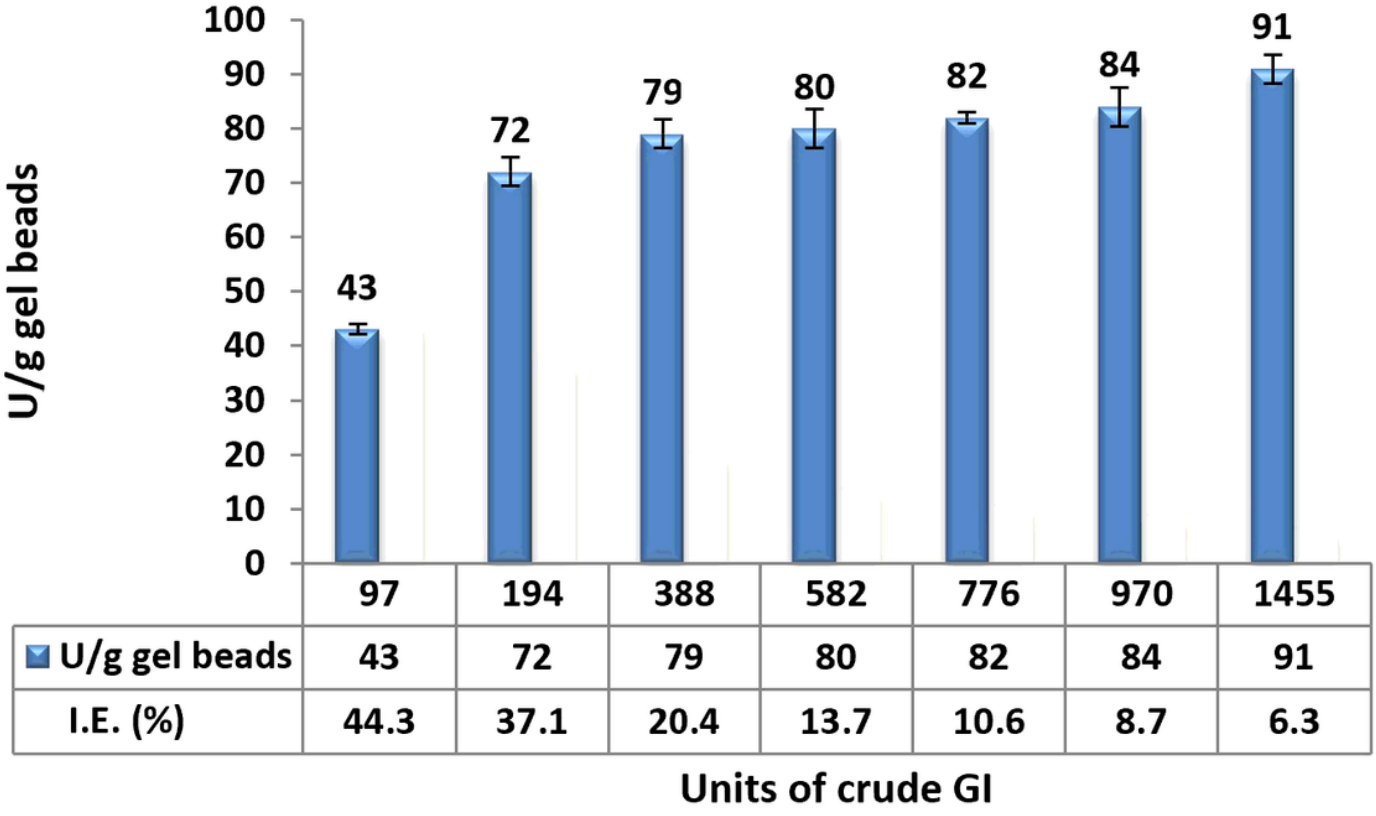

Figure 2

Local GI loading capacity. One gram of treated gel beads was soaked in 97-1455 units of GI solution for 1 h at room temperature.

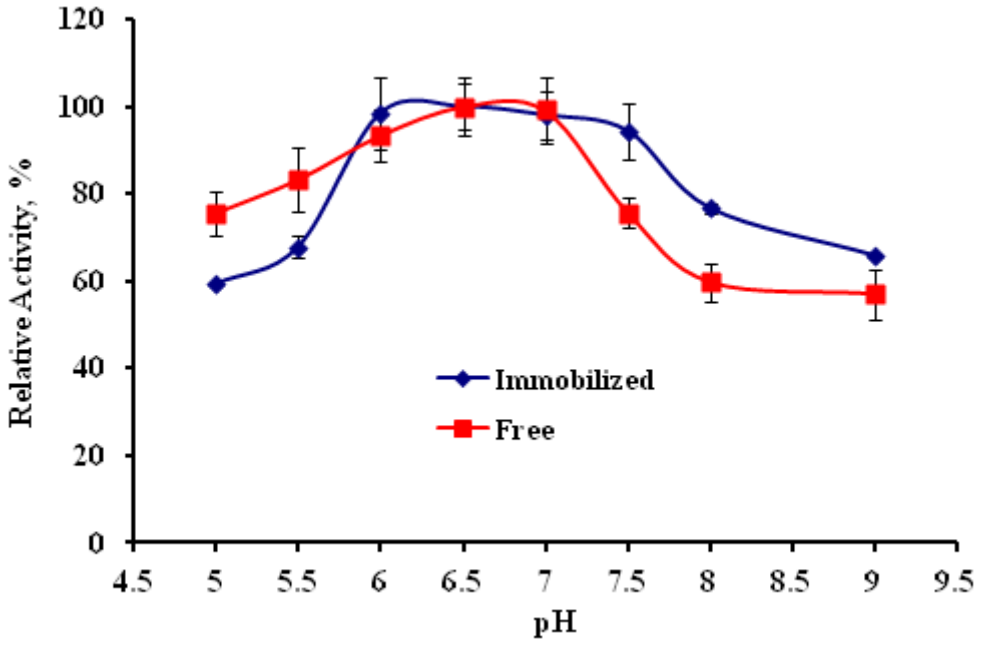

Figure 3

Optimum pH for free and covalently immobilized GI.

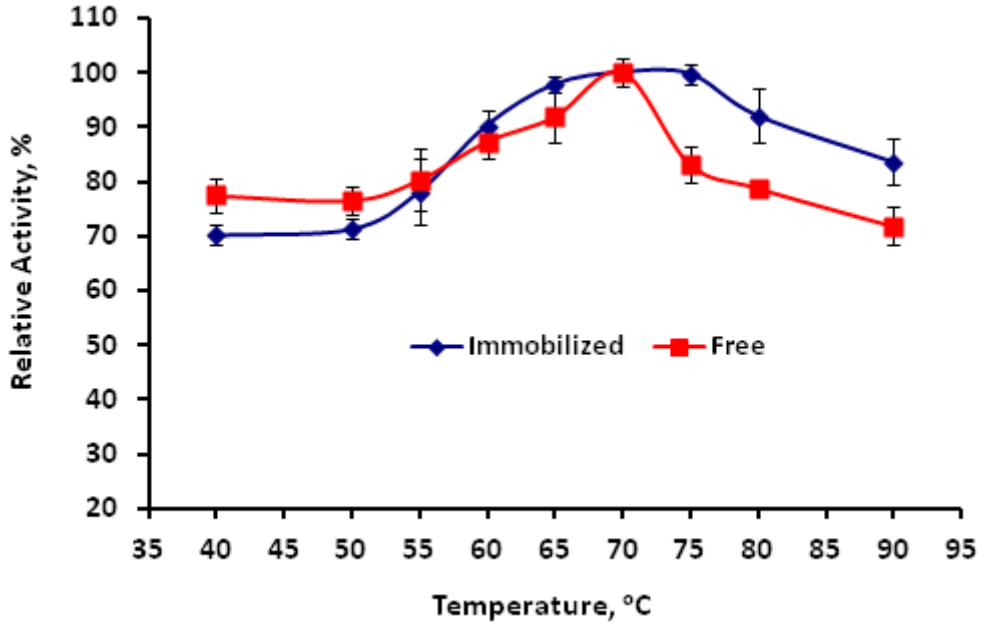

Figure 4

Optimum temperature for free and covalently immobilized GI.

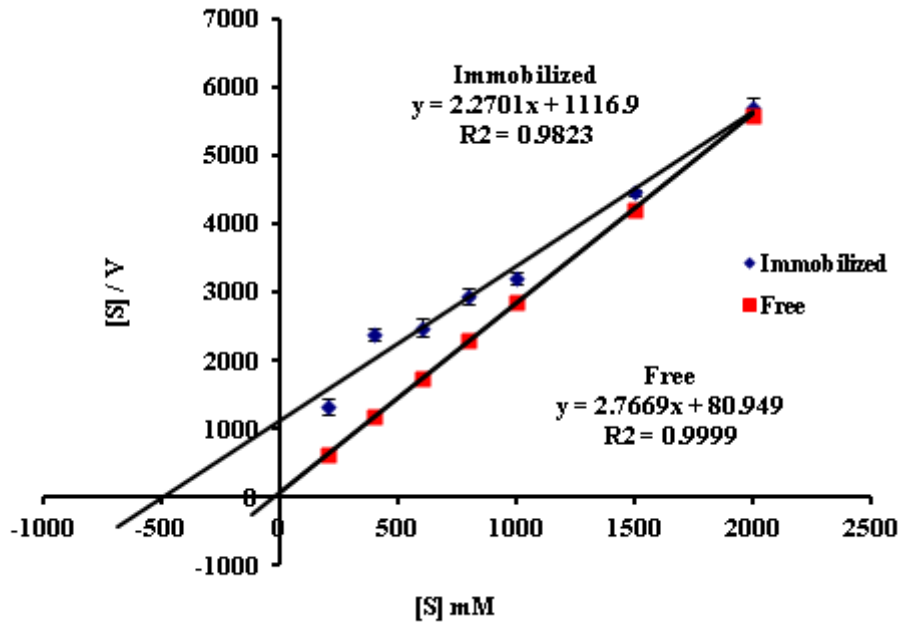

Figure 5

Hanes–Woolf plot of free and immobilized GI.

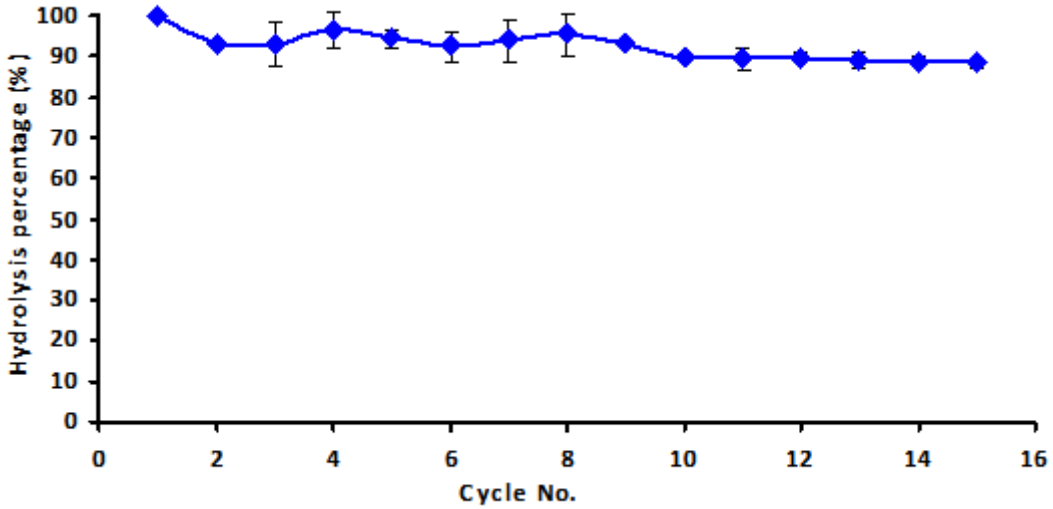

Figure 6

Reusability of immobilized GI.

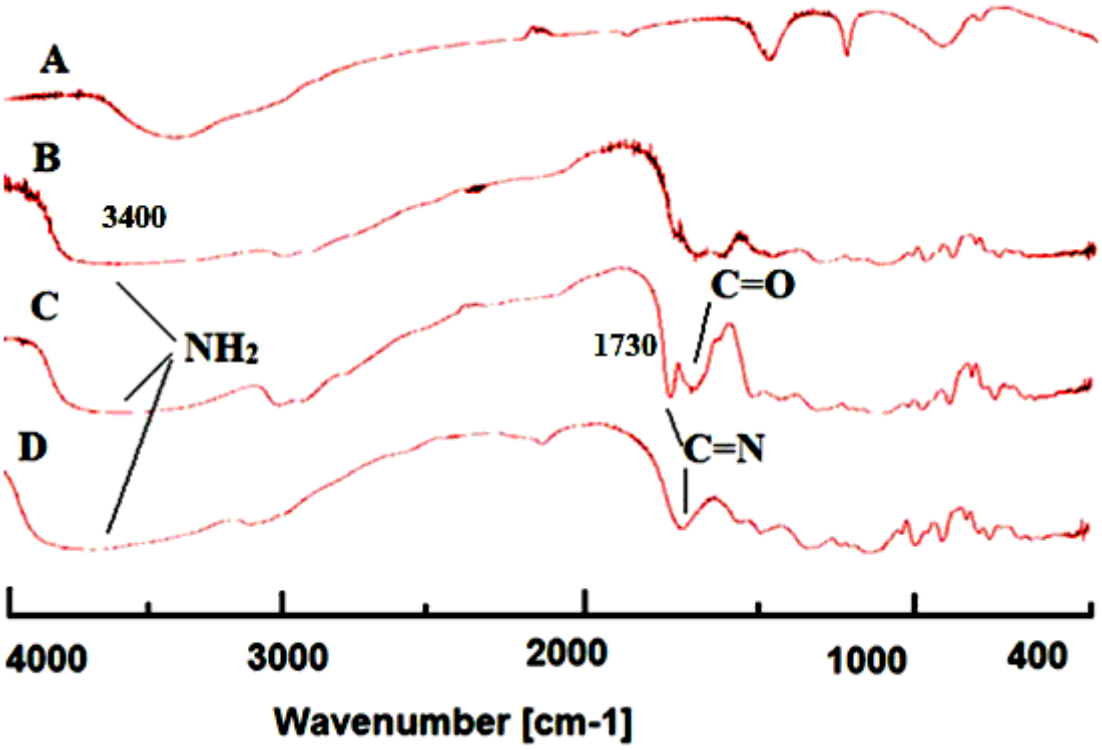

Figure 7

FTIR for κ-carrageenan (A), aminated κ-carrageenan (B), activated κ-carrageenan (C) and enzyme immobilized on κ-carrageenan (D). Figure adapted from Hassan et al., 2019 (open access).

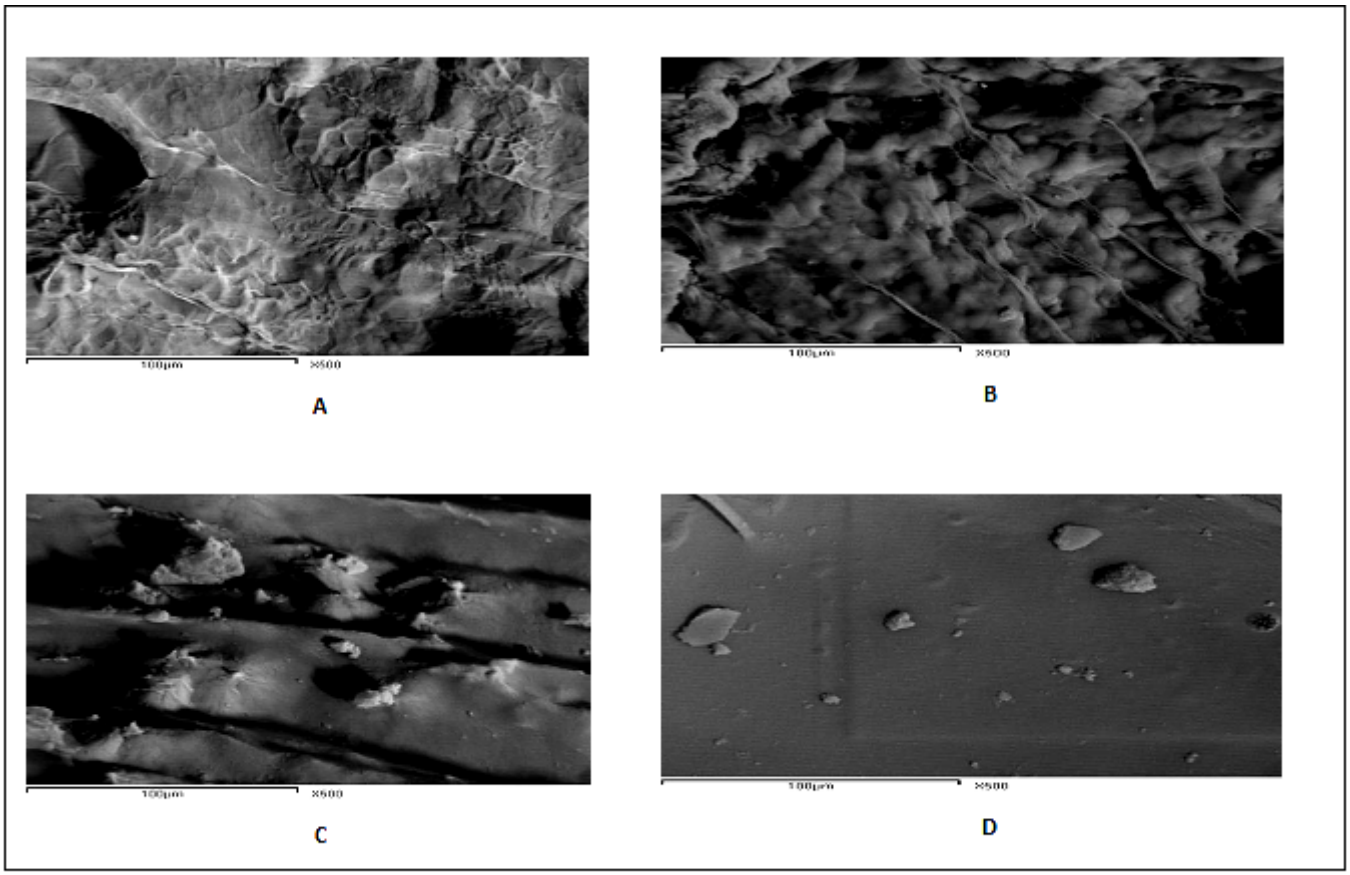

Figure 8

SEM for κ-carrageenan (A), aminated κ-carrageenan (B), activated κ-carrageenan (C) and enzyme immobilized on κ-carrageenan (D). The magnification factor is 500x.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.jpg