**Different spacer-arm attached magnetic nanoparticles for covalent immobilization of Jack bean urease**



**Suplemental Figure 1.** The covalent immobilization scheme of urease onto MNPs via glutaraldehyde (a) and epichlorohydrin (b) spacer arms.





**Suplemental Figure 2.** The storage stability of free and immobilized urease preparations at 4 °C (A), and 25 °C (B) after 30 days storage time.

**Suplemental Table 1.** Comparasion of optimum pH, temperature and termal stability of different studies.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Free form | pH | temperature | Thermal  stability | Immobilized form | pH | temperature | Thermal  stability | References |
| Jack bean urease | 7.0 | 35 °C | All of initial activity loss  after 7 days | Covalent immobilization onto amino functionalized cellulose | 8.0 | 45 °C | 25% of initial activity loss after 7 days | Alatawi et al.  https://doi.org/10.1016/j.ijbiomac.2018.03.142 |
| Canavalia ensiformis  urease | 6.5 | 50 °C | 93% of its initial activity loss after 8 h incubation at 70 °C | Covalent immobilization onto modified zirconium (IV) oxide | 7.0 | 60 °C | 24% of its initial activity loss after 8 h incubation at 70 °C | Alptekin  https://doi.org/10.1007/s11696-021-01891-6 |
| Canavalia ensiformis  urease | 7.5 | 50 °C | %99 of initial activity loss after 4 h incubation at 70 °C | Immobilization in hydrogel matrix | 8.5 | 60 °C | %61 of initial activity loss after 4 h incubation at 70 °C | Kutcherlapati et al.  https://doi.org/10.1016/j.jcis.2015.10.051 |
| Soybean  urease | 7.0 | 60 °C | 60% of its initial activity loss after 5 h incubation at 70 °C | Covalent immobilization on cotton-derived nanocellulose-dialdehyde | 7.0 | 70 °C | 40% of its initial activity loss after 5 h incubation at 70 °C | Tamaddon and Arab  https://doi.org/10.1039/C9RA05240B |
| Jack bean  urease | 7.0 | 40 °C | All of initial activity loss after 80 min incubation at 60 °C | Adsorption on polyvinyl alcohol (PVA)/Chitosan nanofibers | 8.0 | 40 °C | 65% of its initial activity loss after 80 min incubation at 60 °C | Kutlu et al.  https://doi.org/10.1080/10826068.2019.1679175 |
| Jack bean  urease | 7.5 | 50 °C | 71% of its initial activity loss after 15 h incubation at 70 °C | Covalent immobilization on magnetic nanoparticles via glutaraldehyde | 7.5 | 70 °C | 36% of its initial activity loss after 15 h incubation at 70 °C | This study |
| Jack bean  urease | 7.5 | 50 °C | 71% of its initial activity loss after 15 h incubation at 70 °C | Covalent immobilization on magnetic nanoparticles via epichlorohydrin | 7.5 | 70 °C | 44% of its initial activity loss after 15 h incubation at 70 °C | This study |

**Suplemental Table 2.** Thermodynamic parameters for thermal inactivation of free urease, urease@MNPs-Si-Glu and urease@MNPs-ECH.

|  |  |  |  |
| --- | --- | --- | --- |
|  | free urease | urease@MNPs-Si-Glu | urease@MNPs-ECH |
| Ed (kJ/mol) | 29.5 | 36.4 | 34.7 |
| ΔH\* (50 °C) (kJ/mol) | 26.8 | 33.8 | 32.0 |
| ΔG\* (50 °C) (kJ/mol) | 109.5 | 111.9 | 111.3 |
| ΔS\* (50 °C) J/mol/K | -255.9 | -256.7 | -245.4 |
| ΔH\* (60 °C) (kJ/mol) | 26.7 | 33.7 | 31.9 |
| ΔG\* (60 °C) (kJ/mol) | 112.1 | 114.8 | 113.9 |
| ΔS\* (60 °C) J/mol/K | -256.9 | -243.4 | -246.1 |
| ΔH\* (70 °C) (kJ/mol) | 26.6 | 33.6 | 31.8 |
| ΔG\* (70 °C) (kJ/mol) | 114.5 | 116.8 | 116.3 |
| ΔS\* (70 °C) J/mol/K | -256.2 | -242.5 | -246.2 |