

# Studies on the immobilization of bromelain by using magnetic chitosan/Fe<sub>3</sub>O<sub>4</sub> micro/nanoparticles as carriers

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## ABSTRACT

In this study, magnetic chitosan/iron (II, III) oxide composite suspension was prepared by combining chitosan with iron ions. Suspension was washed with water until it was neutralized and then bromelain was added to the suspension. The chitosan/iron (II, III) oxide/bromelain micro/nano particles were produced by low-temperature spray-drying. The larger and smaller particles were collected using an electromagnet collector (EMC) and an electrostatic precipitator (ESP) respectively. Scanning electron microscopy micrographs indicate that the diameters of larger particles collected by EMC were in the range of 500 to 2500 nm while the diameters of smaller particles collected by ESP were in the range of 200 to 1000 nm. Both particles exhibited a rough characteristic. Casein hydrolysis tests were used to determinate the enzyme activities of all the particles. The results indicated that all the micro/nano particles had relative activities higher than 80% and could be reused at least ten times. The relative enzyme activities were increased with increasing chitosan content in the particles. The encapsulation of bromelain with magnetic chitosan/iron (II, III) oxide achieved the goals of enzyme quick recovery and reuse.

Keywords : Magnetic chitosan/iron (II、III) oxide/bromelain micro/nanoparticles、Low-temperature spray-drying、Bromelain

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## REFERENCES

1. 李玉寶、顧寧、魏于全。2006。奈米生醫材料。五南圖書出版公司。
2. 徐世昌。2001。生物高分子-幾丁質與幾丁聚醣之介紹與應用。化工資訊 15 (2):36-45。
3. 郭竹芳。2004。Invertase 固定於 Fe<sub>3</sub>O<sub>4</sub> 磁性奈米載體及其在磁穩性填充床 (MSFBR) 之研究。國雲林科技大学化學工程學系碩士論文:14-16。
4. 傅昭銘。2003。奈米磁顆粒之放射性標化及應用簡介。物理雙月刊25 (3):1-4。
5. 楊謝樂。2006。磁性奈米粒子於生物醫學上之應用。物理雙月刊28 (4):692-697。
6. 莊景光。2004。離子鍵結型奈米微粒製備與其對小腸上皮細胞滲透能力之探討。國立清華大學化學工程學系碩士論文:1-50。
7. 黃世宏。2003。氧化鐵磁性奈米粒子在酵素固定化及分離上之應用。國立成功大學化學工程研究所碩士論文:12-15。
8. 黃新義、?耀國。2009。中華民國專利局。磁性奈米粉末製造及收集設備之結構改良。新型第 M 353785 號。
9. 劉伊郎、陳恭。2000。氧化鐵 (Fe<sub>3</sub>O<sub>4</sub>) 薄膜與超晶格。物理雙月刊22 (6):592-605。
10. 鄭宇書。2008。低溫奈米噴霧乾燥設備開發及應用。大葉大學生物產業科技系碩士論文。
11. Berry, C. C., and Curtis, A. S. G. 2003. Functionalisation of magnetic nanoparticles for application in biomedicine. Journal of Physics D: Applied Physic. 36: 198-206.
12. Carpenter, E. E. 2001. Iron nanoparticles as potential magnetic carriers. Journal of Magnetism and magnetic materials. 255: 17-20.
13. Denkbas, E. B., Kilicay, E., Birlikseven, C., and Ozturk, E. 2002. Magnetic chitosan microspheres: preparation and characterization. Reactive & Functional Polymers. 50: 225-232.
14. Desai, K. G. H., and Park, H. J. 2005. Preparation and characterization of drug-loaded chitosan-tripolyphosphate microspheres by spray drying. Drug Development Research. 64: 114-128.
15. Harrach, T., Eckert, K., Schulze-Forster K., Nuck R., Grunow D., and Maurer H. R. 1995. Isolation and partial characterization of basic proteinases from stem. Journal of Protein Chemistry 14: 41-52.
16. Huang, S. Y., Shieh, Y. T., Shih, C. M., and Twu, Y. K. 2010. Magnetic chitosan/iron (II, III) oxide nanoparticles prepared by spray-drying. Carbohydrate Polymers 81: 906-910.
17. Huber, D. L.

2005. Synthesis properties and applications of iron nanoparticle. *Small*. 5: 482-501. 18. Hong, J., Gong, P., Xu, D., Dong, L., and Yao, S. 2007. Stabilization of -chymotrypsin by covalent immobilization on amine-functionalized superparamagnetic nanogel. *Journal of Biotechnology*. 128: 597-605. 19. Jeong, J. R., Shin, C. S., Lee, S. J., and Kim, J. D. 2005. Magnetic properties of superparamagnetic -Fe<sub>2</sub>O<sub>3</sub> nanoparticles prepared by coprecipitation technique. *Journal of Magnetism and Magnetic Materials*. 286: 5-9. 20. Kim, E. H., Ahn, Y., and Lee, H. S. 2007. Biomedical applications of superparamagnetic iron oxide nanoparticles encapsulated within chitosan. *Journal of Alloys and Compounds*. 434/435: 633-636. 21. Lin, Y. H., Mi, F. L., Chen, C. T., Chang, W. C., Peng, S. H., Liang, H. F. and Sung, H. W. 2007. Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. *Biomacromolecules* 8: 146-152. 22. Nguyen, T. T. B., Hein, S., Ng, C. H., and Stevens, W. F. 2007. Molecular stability of chitosan in acid solutions stored at various conditions. *Journal of Applied Polymer Science*. 107: 2588-2593. 23. Okuyama, K., Noguchi, K., Kanenari, M., Egawa T., Osawa, K., and Ogawa, K. 2000. Structural diversity of chitosan and its complexes. *Carbohydr. Polymers*. 41: 237-247. 24. Rowan, A. D, Buttle D. J, and Barrett, A. J. 1990. The cysteine proteinases of the pineapple plant. *Biochemistry*: 266: 869-875. 25. Sun, K. Y., Ma, M., Zhang, Y., and Gu, N. 2004. Synthesis of nanometer-size maghemite particles from magnetite. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 245: 15-19. 26. Wang, Z. L., Finlay, W. H., Peppler, M. S., and Sweeney, L. G. 2006. Powder formation by atmospheric spray-freeze-drying. *Powder Technology* 170: 45-52. 27. White, R. R., Crawley, F. E., Vellini, M., and Rovati L. A. 1988. Bioavailability of 125I bromelain after oral administration to rats. *Biopharmaceutics & Drug Disposition*. 9: 397-403. 28. Wu, Y., Guo, J., Yang, W., Wang, C., and Fu, S. 2006. Preparation and characterization of chitosan-poly(acrylic acid) polymer magnetic microspheres. *Polymer*. 47: 5287-5294. 29. Xiaoyan, A., Jun, Y., Min, W., Haiyue, Z., Li, C., Kangde, Y., and Fanglian, Y. 2008. Preparation of chitosan-gelatin scaffold containing terandrine-loaded nano-aggregates and its controlled release behavior. *International Journal of Pharmaceutics* 350: 257-264. 30. Yan, G. P., Robinson, L., and Hogg, P. 2007. Magnetic resonance imaging contrast agents: Overview and perspectives. *Radiography*. 13: 5-19. 31. Yu, Z., Rogers, T. L., Hu, J., Johnston, K. P., and Williams, R. O . 2002. Preparation and characterization of microparticles containing peptide produced by a novel process: spray freezing into liquid. *European Journal of Pharmaceutical and Biopharmaceutics*. 54: 221-228. 32. Zhou, H. Y., Chen, X. G., Meng, X. H., Lin, C. G., Yu, L. J. 2006. Release characteristics of three model drugs from chitosan/cellulose acetate multimesosphere. *Biochemical Engineering Journal* 31: 228-233. 33. Zhu, A., Yuan, L. and Liao, T. 2008. Suspension of Fe<sub>3</sub>O<sub>4</sub> nanoparticles stabilized by chitosan and o-carboxymethylchitosan. *International Journal of Pharmaceutics*. 350: 361-368.