

# 奈米幾丁聚醣藥物載體之製備及應用研究=Preparation and application of Nano Chitosan drug carriers

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## 摘要

本研究目的在製備出奈米級的幾丁聚醣藥物載體，首先將市售幾丁聚醣溶於鹽酸中，並調整成適當之酸鹼值，乾燥後便得可溶於水的幾丁聚醣鹽酸鹽。再將幾丁聚醣鹽酸鹽混和不同濃度之架橋劑-綠梔子素與消炎止痛藥-待克菲那配製成溶液，進行噴霧乾燥得到奈米幾丁聚醣/待克菲那藥物載體，並對產物作粒徑及物性分析、細胞毒性、穿透實驗及藥物釋放之評估。由場發射電子顯微鏡觀察，各樣本平均粒徑約在300 nm。利用富立葉紅外線光譜儀偵測發現，樣本中含有藥物時，在1456 cm<sup>-1</sup>處出現特徵吸收，且當架橋劑濃度增加時，在1540 cm<sup>-1</sup>之胺基吸收峰減小，顯示幾丁聚醣鹽酸鹽顆粒中有藥物待克菲那的存在，與架橋劑有架橋反應生成。將藥物載體與腸道上皮細胞(Caco-2)進行細胞毒性及穿透評估，發現藥物載體在濃度100 µg/mL以下對Caco-2細胞無明顯毒性。而由TEER實驗結果發現，樣本D有較佳的穿透能力達50%，若樣本保存不善造成吸濕，顆粒變大則無法順利通過細胞間隙，細胞經過trypan blue染色後發現，樣本並未對細胞造成傷害。由藥物釋放研究發現，在前1小時小於50%附著於表面的待克菲那會先被釋放出來，且隨時間增長藥物濃度也逐漸上升，因此確認幾丁聚醣藥物載體具有緩釋藥物之能力。

關鍵詞：幾丁聚醣；藥物載體；噴霧乾燥；藥物釋放

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- 1.甘霖。2004。奈米投藥系統在標地給藥上的應用。化工資訊與商情13:48-49。
- 2.行政院衛生署。2000。中華藥典第五版。第431-432頁。
- 3.李安榮、鄒台黎、黃文鑫。2005。新編藥物學。第(2)14-(2)16頁。永大出版社。台北，台灣。
- 4.邱少華。2000。幾丁聚醣在藥物釋放上的應用以及其微膠囊製備的技術。生物資源 生物技術2(3):19-23。
- 5.施詔銘、?耀國。2005，奈米粉末製造機之結構與改良。中華明國新型專利第M27339號。
- 6.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。 7.袁國芳、林欣榜、賴進此、陳慶源。2001。幾丁質/幾丁聚醣專輯。第1-164頁。食品工業發展研究所。新竹，台灣。 8.莊仲揚、陳俊男。2006。幾丁聚醣於生醫產業上的應用。化工資訊與商情38:63-66。 9.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:1-50。清華大學化學工程學系碩士論文。新竹，台灣。 10.陳俐婷。2002。脈衝列電磁場刺激對骨母細胞生物活性的影響:14-15。中原大學醫學工程研究所碩士論文。中壢，台灣。 11.陳嘉芬。2002。細胞生物學。第301-307頁。藝軒出版社。台北，台灣。 12.陳慶源。2000。幾丁聚醣在藥物運輸系統上之應用。食品工業32 ( 4 ) :18-28。 13.葉紹任等。2005。隱藏性耐米高分子微胞的藥物輸送應用。化工資訊與商情26:60-65。 14.蔡宏銘。2002。中藥對骨細胞活性的評估:7-27。中國醫藥學院中國醫學研究所碩士論文。台中，台灣。 15.魏育慧。2000。利用動物細胞進行抗癌藥物篩選。食品工業32(11) :27-35。 16.顧寧、付德剛、張海黔。2003。奈米技術與應用。第191-206頁。滄海出版社。台中，台灣。 17.Aksungur, P., Sungur, A., Unal, S., ?skit, A. B., Squier, C. A. and ?enel, S. 2004. Chitosan delivery systems for the treatment of oral mucositis:in vitro and in vivo studies. *Journal of Controlled Release*. 98:269-279.
- 18.Alpar, H. O., Somavarapu, S., Atuah, K. N. and Bramwell. 2005. Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery. *Advanced Drug Delivery Reviews*. 57:411-430. 19.Asada, M., Takahashi, H., Okamoto, H. and Danjo, K. 2004. Theophylline particle design using chitosan by the spray drying. *International Journal of Pharmaceutics*. 270:167-174. 20.Boonsongnirt, Y., Mitrevej, A. and Mueller, B. W. 2006. Chitosan drug binding by ionic interaction. *European Journal of Pharmaceutics and Biopharmaceutics*. 62:267-274.
- 21.Cevher, E., Orhan, Z., Mulaz?mo?lu, L., ?ensoy, D., Alper, M., Y?ld?z, A. and Ozsoy, Y. 2006. Characterization of biodegradable chitosan microspheres containing vancomycin and treatment of experimental osteomyelitis caused by methicillin-resistant *Staphylococcus aureu* with prepared microspheres. *International Journal of Pharmaceutics*. 317:127-135. 22.Chen, S. C., Wu, Y. C., Mi, F. L., Lin, Y. H., Yu, L. C. and Sung, H. W. 2004. A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. *Journal of Controlled Release*. 96:285-300. 23.Corrigan, D. O., Healy, A. M. and Corrigan, O. I. 2006. Preparation and release of salbutamol from chitosan and chitosan co-spray dried compacts and mutiparticulates. *European Journal of Pharmaceutics and Biopharmaceutics*. 62:295-305. 24.Eirheim, H. U., Bundaard, C. and Nielsen, H. M. 2004. Evaluation of different toxicity assays applied to proliferating cells and to stratified epithelium in relation to permrability enhancement with glycocholate. *Toxicology in Vitro*. 18:649-657. 25.Florea, B. I., Thanou, M., Junginger, H. E. and Borchard, G. 2006. Enhancement of bronchial octreotide absorption by chitosan and N-trimethyl chitosan shows linear in vitro/in vivo correlation. *Journal of Controlled Release*. 110:353-361. 26.Gonzalez-Rodriguez, M. L., Holgado, M. A., Sanchez-Lafuente, C., Rabasco, A. M. and Fini, A. 2002. Alginate/chitosan particulate systems for sodium diclofenac release. *International Journal of Pharmaceutics*. 232:225-234. 27.Grenha, A., Seijo, B. and Remunan-Lopze, C. 2005. Microencapsulated chitosan nanoparticles for lung protein delivery. *European Journal of Pharmaceutical Sciences*. 25:427-437. 28.Gupta, K. C. and Kumar, M. R., 2000. Drug release behavior of beads and microgranules of chitosan. *Biomaterials*. 21:1115-1119. 29.Hsieh, C. Y., Tsai, S. P., Ho, M. H., Wang, D. M., Liu, C. E., Hsieh, C. H., Tseng, H. C. and Hsieh, H. J. 2007. Analysis of freeze-gelation and cross-linking processes for preparing porous chitosan scaffolds. *Carbohydrate Polymers*. 67:124-132. 30.Huang, Y. C., Yeh, M. K. and Chiang, C. H. 2002. Formulation factors in preparing BTM-chitosan microspheres by spray drying method. *International Journal of Pharmaceutics*. 242:239-242. 31.Jia, Z., Yujun, W., Yangcheng, L., Jingyu, M. and Guangsheng, L. 2006. Reactive & Functional Polymers. 66:1552-1558. 32.Kim, J. C., Lee, H. Y., Kim, M. H., Lee, H. J., Kang, H. Y. and Kim, S. M. 2006. Preparation and characterization of chitosan/gelatin microcapsules containing triclosan. *Colloids and Surfaces B:Biointerfaces*. 52:52-56. 33.Kockisch, S., Rees, G. D., Tsibouklis, J. and Smart, J. D. 2005. Mucoadhesive, triclosan-loaded polymer microspheres for application to the oral cavity:preparation and controlled release characteristics. *European Journal of Pharmaceutics and Biopharmaceutics*. 59:207-216. 34.Liu, D. Z., LeCluyse, E. L. and Thakker, D. R., 1999. Dodecylphosphocholine-mediated enhancement of paracellular permeability and cytotoxicity in Caco-2 cell monolayers. *Journal of Pharmaceutical Sciences*. 88(11):1161-1168. 35.Maestrelli, F., Garcia-Fuentes, M., Mura, P. and Alonso. M. J. 2006. European Journal of Pharmaceutics and Biopharmaceutics. 63:79-86. 36.Mi, F. T., Wong, T. and Shyu, S., 1999. Chitosan microspheres:modification of polymeric chemphysical properties of spray-dried microspheres to control the release of antibiotic drug. *Journal of Applied Polymer Science*, 71:747-759.
- 37.Mi, F. L., Sung, H. W. and Shyu, S. S. 2002a. Drug release from chitosan-alginate complex beads reinforced by a naturally occurring cross-linking agent. *Carbohydrate polymers*, 48:61-72. 38.Mi, F. L., Tan, Y. C., Liang, H. F. and Sung, H. W. 2002b. In vivo biocompatibility and degradability of novel injectable-chitosan-base implant. *Biomaterials*. 23:181-191. 39.Muzzarelli, C., Stanic, V., Gobbi, L., Tosi, G. and Muzzarelli, R. A. A. 2004. Spray-drying of solutions containing Chitosan together with polyuronans and characterization of the microspheres. *Carbohydrate polymers*. 57:73-82. 40.Nunthanid, J., Laungana-anan, M., Sriamornsak, P., Limmatvapirat, S., Puttipipatkhachorn, S., Lim, L. Y. and Khore, E. 2004. Characterization of Chitosan acetate as a binder for sustained release tablets. *Journal of Controlled Release*. 99:15-26.
- 41.Olivier, P., Testard, P., Marzin, D. and Abbott, D. 1995. Effect of High polyol concentrations on the neutral red absorption assay and tetrazolium-MTT test of rat hepatocytes in primary culture. *Toxicology in Vitro*. 9(2):133-138. 42.Orienti, I., Cerchiara, T., Luppi, B., Bigucci, F., Zuccari, G. and Zecchi, V. 2002. Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. *International Journal of Pharmaceutics*. 238:51-59. 43.Prego, C., Garcia, M., Torres, D. and Alonso, M. J. 2005. Transmucosal macromolecular drug delivery. *Journal of Controlled Release*. 101:151-162. 44.Rege, P. R., Garmise, R. J. and Block, L. H. 2003. Spray-dried chitosans Part :preparation and characterization. *International Journal of Pharmaceutics*. 252:41-51. 45.Sava?er, A., Ozkan, Y. and I??mer, A. 2005. Preparation and in vitro evaluation of sustained release tablet formulations of diclofenac sodium. II *Farmaco*. 60:171-177. 46.Sandri, G., Bonferoni, M. C., Rossi, S., Ferrari, F., Gibin, S., Zambito, Y., Colo, G. D. and Caramella, C. 2007. Nanoparticles based on N-trimethylchitosan:Evaluation of absorption properties using in vitro ( Caco-2 cells ) and ex vivo ( excised rat jejunum ) models. *European Journal of Pharmaceutics and Biopharmaceutics*.

65:68-77. 47.Sankararamakrishnan, N., Dixit, A., Iyengar, L. and Sanghi. R. 2006. Removal of hexavalent chromium using a novel cross linked xanthated chitosan. *Bioresource Technology*. 97:2377-2382. 48.Thanou, M., Henderson, S., Kydonieus, A. and Elson, C. 2007. N-sulfonsto-N,O-carboxymethylchitosan:A novel polymeric absorption enhancer for the oral delivery of macromolecules. *Journal of Controlled Release*. 117:171-178. 49.Varum, K. M., Egelalndal, B. and Ellekjar, M. R. 1995. Characterization of partially N-acetylated chitosans by near infra-red spectroscopy. *Carbohydrate Polymers*. 28:187-193. 50.Walgren, R. A., Walle, U. K. and Walle, T. 1998. Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. *Biochem. Pharmacol.* 55:1721-1727. 51.Wang, L. Y., Ma, G. H. and Su, Z. G., 2005. Preparation of uniform size chitosan microspheres by membrane emulsification technique and application as a carrier of protein drug. *Journal of Controlled Release*. 106:62-75. 52.Zerrouk, N., Corti, G., Ancillotti, S., Maestrelli, F., Cirri, M. and Mura, P. 2006. Influence of cyclodextrins and chitosan, separately or in combination on glyburide solubility and permeability. *European Journal of Pharmaceutics and Biopharmaceutics*. 62:241-246 53.Zhang, W. F., Chen, X. G., Li, P. W., He, Q. Z. and Zhou, H. Y. 2007. Chitosan and Chitosan/  $\alpha$ -Cyclodextrin Microspheres as Sustained-Release Drug Carriers. *Journal of Applied Polymer Science*. 103:1183-1190. 54.Zhao, A., Yao, P., Kang, C., Yuan, X., Chang, J. and Pu, P. 2005. Synthesis and characterization of tat-mediated O-CMC magnetic nanoparticles having anticancer function. *Journal of Magnetism and Magnetic Materials*. 295:37-43.