

Preparation and Application of Nano Chitosan Drug Carriers

曾詣文、

E-mail: 9607601@mail.dyu.edu.tw

ABSTRACT

The objective of this study was to prepare nano chitosan drug carriers. First, chitosan was dissolved in HCl and the pH of solution was adjusted. Chitosan hydrochloride was obtained after dehydration. The solutions of chitosan-HCl with diclofenac sodium and genipin were prepared. Spray-drying was used to prepare nano chitosan drug carriers containing diclofenac sodium. To evaluate characteristics of these carriers, FESEM, cytotoxicity, permeability, and drug release tests were conducted. The FESEM indicated that the size of carriers had an average size of 300 nm. The FTIR indicated that the samples appeared characteristic absorption peak when containing drugs. When increased the concentration of cross-linking agent in these carriers, the IR absorption peak of amide was decrease. MTT data showed that these carriers with a concentration below 100 ug/mL were non-toxic to Caco-2 cells. In TEER results, sample D has a penetration greater than 50 %. When samples containing moisture, aggregation happened and the size of samples become larger. Hence samples can't penetrate cell junction successfully. The results of trypan blue assay showed that samples won't harm the cells. The drug release behaviors showed that less than 50 % diclofenac sodium could be release in one hour. With prolonging releasing time, the drug concentration was increasing. Chitosan drug carriers have potential in the application of controlled release.

Keywords : chitosan ; drug carriers ; spray drying ; drug release

Table of Contents

封面內頁 簽名頁 授權書.....	iii 中文摘要.....	iv 英文摘要.....	v 誌
謝.....	vi 目錄.....	vii 圖目錄.....	x 表目
錄.....	xii 1. 研究目的.....	1 2. 文獻回顧.....	2 2.1 幾丁質與幾丁聚醣簡
介.....	2 2.2 幾丁聚醣鹽類.....	2 2.3 架橋劑綠梔子素 (genipin) 簡介.....	4 2.4 待克菲那
(diclofenac sodium) 簡介.....	7 2.5 幾丁聚醣於藥物控制釋放.....	9 2.5.1 幾丁聚醣藥物載體的特性.....	11
2.6 奈米化藥物載體.....	13 2.6.1 噴霧乾燥法.....	14 2.7 口服藥物傳輸模式.....	16
2.7.1 Caco-2單層膜細胞.....	16 2.7.2 幾丁聚醣物質於腸道穿透之研究.....	17 2.8 細胞毒性評	
估.....	20 2.8.1 細胞毒性試驗方法.....	20 2.9 藥物釋放評估.....	22 2.9.1 分光光度計偵
測法.....	22 2.9.2 HPLC定量法.....	22 3. 材料與方法.....	23 3.1 實驗架
構.....	23 3.2 實驗材料.....	24 3.2.1 藥品.....	24 3.2.2 耗
材.....	25 3.2.3 儀器設備.....	26 3.2.4 細胞株.....	27 3.3 研究方
法.....	28 3.3.1 幾丁聚醣鹽酸鹽製備.....	28 3.4 幾丁聚醣鹽酸鹽藥物載載體製備.....	28 3.4.1
噴霧乾燥法製備奈米藥物載體.....	30 3.5 產物分析.....	30 3.5.1 場發射電子顯微鏡 (FESEM)	
).....	30 3.5.2 富立葉紅外線光譜儀試驗 (FTIR)	31 3.6 細胞毒性之研究.....	31 3.6.1 細胞復
甦.....	31 3.6.2 繼代培養.....	32 3.6.3 MTT 毒性試驗.....	34 3.7 細胞穿透之研
究.....	34 3.7.1 TEER試驗.....	35 3.7.2 Trypan blue細胞存活率試驗.....	35 3.8 藥物釋放試
驗.....	37 3.8.1 標準品配製.....	37 3.8.2 藥物釋放分析.....	37 4. 結果與討
論.....	38 4.1 幾丁聚醣鹽酸鹽及載體之製備.....	38 4.2 場發射電子顯微鏡觀察.....	39 4.3 富
立葉紅外線光譜儀試驗 (FTIR)	52 4.4 細胞試驗.....	57 4.4.1 Caco-2細胞培養觀察.....	57
4.4.2 細胞毒性試驗 (MTT分析)	58 4.4.3 Transepithelial electrical resistance (TEER) 分析.....	68 5. 結論.....	59
4.4.4 Trypan blue存活率試驗.....	61 4.5 藥物釋放試驗.....	74 參考文	
獻.....	76		

REFERENCES

- 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.衛生署。台北，台灣。
- 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.Boonsongkirt, 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. *Il 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/ -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.