

Optimization of preparation condition for glabridin nanoemulsion by collision-high pressure homogenization

盧意淇、柯文慶、謝昌衛

E-mail: 322032@mail.dyu.edu.tw

ABSTRACT

Nanoemulsion droplets size is in the range of 10-100 nm. Nanoemulsion was stable and easily absorbed through skin due to its miniaturized size. High pressure homogenization may be applicable to manufacture nanoemulsion products by providing great energy in a short time with a homogeneous flow to decrease particle size. In this study, the effects of oil (2-6%, w/w), surfactant (3-7%, w/w), and the homogenization pressure (70-130 MPa) on the particle size of emulsion were investigated using response surface methodology (RSM) with 3-factor-3-level Box-Behnken design. Based on the analysis of canonical, the effect of the number of passes of homogenization on particle size to obtain emulsion with low particle size and high stability was determined. Then, glabridin was added to the nanoemulsion as a functional ingredient, and effectiveness of the product was evaluated. Results showed that a good nanoemulsion was obtained by mixing oil 3.65% with emulsifier 5.3% and then homogenized at 129 MPa. The actual minimum particle size (58 nm) was closed to the predicted value (40.4 nm). Homogenization pressure was the key factor affecting particle size from the analysis of variance for joint test ($p < 0.005$). The particle size was proportionally reduced as the pressure increased. Moreover, the number of passes of homogenization could also affect the particle size, and stable particle size at about 28 nm was resulted after 3 passes of homogenization. Glabridin had a high DPPH radical scavenging activity (80%) of 1 mg/mL. Activity of tyrosinase was inhibited by 80% using 1 μ g/mL of glabridin. The nanoemulsion containing glabridin retained tyrosinase inhibitory activity even after high pressure homogenization. The cumulative amount (0.086 g/mL) for the product was better than that that of non-high pressure homogenization (0.057 mg/mL) after transdermal diffusion for 24 hours. Furthermore, the glabridin nanoemulsion did not cause any irritation or allergy during the safety and stability test. In conclusion, this study showed that an optimal condition was developed for the preparation of a stable nanoemulsion, and glabridin is a highly valuable active ingredient for whitening the skin. Results also showed that the miniaturized particle of this product could be absorbed without irritation and allergy through skin while providing an effective high value skin care.

Keywords : Glabridin、Nanoemulsion、Collision-high pressure homogenization、Response surface methodology

Table of Contents

封面內頁 簽名頁 授權書 iii 中文摘要 iv 英文摘要 vi 誌謝 viii 目錄 ix 圖目錄 xiii 表目錄 xvi 1. 前言 1 2. 文獻回顧 3 2.1 化妝品的定義與種類 3 2.2 皮膚與黑色素的生成 5 2.2.1 自由基、抗氧化與美白 8 2.2.2 常見美白成分介紹 8 2.3 甘草黃酮類成分-光甘草定 9 2.4 乳液 10 2.4.1 乳化作用 10 2.4.2 乳化安定性 14 2.5 奈米乳液 18 2.5.1 奈米乳液的類型 19 2.5.2 奈米乳化的動力學穩定性 19 2.5.3 奈米乳液的製備 23 2.5.4 奈米乳液的應用優勢 23 2.6 界面活性劑 25 2.6.1 界面活性劑的分類 28 2.6.2 HLB 值 29 2.7 高壓均質技術機制與應用 30 2.8 經皮吸收 32 3. 材料與方法 37 3.1 材料與儀器 37 3.1.1 藥品與材料 37 3.1.2 實驗儀器 38 3.1.3 高壓均質系統操作流程 39 3.2 實驗設計與流程 43 3.3 實驗方法 43 3.3.1 微乳液區試驗 43 3.3.2 製備製備奈米乳液條件探討 44 3.3.3 奈米乳液的製備 44 3.3.3.1 奈米乳液粒徑檢測 44 3.3.3.2 高壓均質法製備奈米乳液最適化條件 45 3.3.4 反應曲面統計分析 45 3.3.5 乳液的品質檢測 49 3.3.5.1 乳液的色澤測定 49 3.3.5.2 乳液的pH值測定 49 3.3.5.3 乳液的微生物試驗 49 3.3.5.4 乳液的乳化安定性 50 3.3.5.5 乳液的熱穩定性 50 3.3.6 Glabridin 生物活性試驗 51 3.3.6.1 Glabridin 之還原力測定 51 3.3.6.2 Glabridin 之亞鐵離子螯合能力測定 51 3.3.6.3 Glabridin 之 DPPH 清除能力測定 51 3.3.6.4 Glabridin 之體外抑制酪胺酸？“壼樟” 52 3.3.6.5 Glabridin 濾紙擴散試驗 52 3.3.7 Glabridin 奈米乳液性有效性試驗 53 3.3.7.1 Glabridin 奈米乳液皮膚累積滲透量 53 3.3.7.2 Glabridin 奈米乳液體外抑制酪胺酸？“壼樟” 55 3.3.8 Glabridin 奈米乳液安全貼布試驗 56 3.3.9 統計分析 56 4. 結果與討論-奈米乳液載體之製備 59 4.1 製備奈米乳液條件探討 59 4.2 高壓均質技術製備奈米乳液反應曲面分析 60 4.3 高壓均質技術製備奈米乳液最適化條件 76 4.4 不同高壓均質壓力和處理次數對乳液色差之影響 77 4.5 不同高壓均質處理次數對乳液粒徑之影響 84 4.6 奈米乳液之微生物試驗 87 4.7 奈米乳液之熱穩定性及安定性試驗 87 5. 結果與討論-Glabridin 奈米乳液之有效性評估 93 5.1 Glabridin 還原力、螯合亞鐵能力、清除 DPPH 能力 93 5.2 Glabridin 奈米乳液體外抑制酪胺酸？“樟” 94 5.3 Glabridin 奈米乳液微生物檢測 102 5.4 Glabridin 奈米乳液熱穩定性及安定性試驗 103 5.5 Glabridin 奈米乳液安全貼布試驗 108 5.6 Glabridin 經皮吸收試驗 108 6. 結論 113 參考文獻 114 圖1. 皮膚美白的作用機轉 7 圖2. 光甘草定結構式 11 圖3. 乳液的形態：油中水滴型乳液、水中油滴型乳液 13 圖4. 乳液不安定示意圖 17 圖5. O/W奈米乳液 20 圖6. 界面活性劑結構圖 27 圖7. 傳統製程化妝品經皮吸收示意圖 35 圖8. 奈米技術製程化妝品經皮吸收示意圖 36 圖9. 高壓

均質系統裝置 40 圖10. 射流對撞式高壓均質閥構造與作用圖 41 圖11. 高壓均質系統處理流程圖 42 圖12. 第一部分實驗架構流程圖-奈米乳液載體製備 46 圖13. 第二部份實驗架構流程圖-光甘草定奈米乳液功能性檢測 47 圖14. 經皮吸收系統擴散槽 54 圖15. 高壓均質技術所製備奈米乳液之粒徑與預測值之相關性 71 圖16. 添加不同濃度的乳化劑及壓力對乳液粒徑影響之反應曲面圖，固定油相比例(a)2%(b)4%(C)6% 73 圖17. 添加不同濃度的油相及壓力對乳液粒徑影響之反應曲面圖，固定處理壓力(a)70 MPa(b)100 MPa(C)130MPa 75 圖19. 添加不同濃度的乳化劑及不同高壓均質處理壓力之下，乳液粒徑變化之等高線圖；固定油的濃度為(a)2%(b)4%(c)6% 78 圖20. 添加不同濃度的油相及不同高壓均質處理壓力之下，乳液粒徑變化之等高線圖；固定乳化劑用量為(a)3%(b)5%(c)7% 79 圖21. 添加不同濃度的油相及乳化劑之下，乳液粒徑變化之等高線圖；固定高壓均質壓力為(a)70 MPa(b)100 MPa(c)130 MPa 80 圖22. 不同高壓均質壓力對奈米乳液之 L₁?彌P WI ?彼v響 81 圖23. 不同高壓均質處理次數對奈米乳液之 L₁?屆B WI?彼v響 82 圖24. 不同高壓均質處理次數的奈米乳液對照圖 83 圖25. 奈米乳液粒徑圖(油相濃度 3.65%、乳化劑濃度 5.3%、高壓均質壓力為 129MPa、高壓均質處理次數 3 次) 86 圖26. Glabridin 與 BHA 之還原力比較 96 圖27. Glabridin 與 EDTA 之螯合亞鐵能力比較 97 圖28. Glabridin 與 BHA 之 DPPH 清除能力比較 98 圖29. 熊果??攜~抑制酪胺酸？“嬸梓 99 圖30. Glabridin 體外抑制酪胺酸？”“嬸梓 100 圖31. Glabridin 奈米乳液體外抑制酪胺酸？ 梓 101 圖32. Glabridin 奈米乳液累積滲透量 112 表1. 乳液的外觀與液滴大小及光學性質的關係 21 表2. 各式均質乳化方法比較 26 表3. 壓力換算表 33 表4. 三階層三變數 Box-Behnken 實驗設計 48 表5. 貼布試驗積分表 57 表6. 貼布試驗平均積分值 58 表7-1 乳液其油水比及界面活性劑對油的比例 64 表7-2 乳液其油水比及界面活性劑對油的比例 65 表8. 不同 HLB 值對乳液粒徑之影響 66 表9. 不同油添加量 (3%、5%、7%、9%、11%) 對乳液粒徑之影響 67 表10. 不同高壓均質處理 (30、50、70、90、110、130 MPa) 對乳液粒徑之影響 68 表11. 利用高壓均質技術所製備乳液粒徑之實驗值與預測值 69 表12. 利用高壓均質技術製備奈米乳液其粒徑對合成變數之變異數分析 70 表13. 利用高壓均質技術製備奈米乳液其粒徑之聯合檢測分析 72 表14. 不同高壓均質處理次數對乳液粒徑之影響 85 表15. 乳液經由不同次數高壓均質處理後其微生物生長之情形 90 表16. 乳液熱穩定性試驗 91 表17. 乳液安定性試驗 92 表18. Glabridin 對Staphylococcus epidermidis (BCRC 11030)、Escherichia coli (BCRC 10450)、之抑菌圈大小 104 表19. Glabridin 奈米乳液經由高壓均質處理後其微生物生長之情形 105 表20. Glabridin 乳液熱穩定性試驗 106 表21. Glabridin 乳液安定性試驗 107 表22. Glabridin 奈米乳液安全貼布測試積分表 110 表23. Glabridin 奈米乳液安全貼布測試平均積分 111

REFERENCES

- 毛立科、許洪高、高彥祥。2007。高壓均質技術與食品乳狀液。食品與機械 23(5): 146-149。
- 木合布力 . 阿布力孜、熱娜 卡斯木、馬淑燕、Vincent loppinet、G?臆ard siest。2007。甘草中光甘草定的提取和抗氧化活性研究。天然產物研究與開發 19(4): 675-677 , 682。
- 王輔男。2005。含美白成分奈米乳化產品之製備及其性質研究。嘉南藥理科技大學化妝品科技研究所碩士論文。嘉義。台灣。4.王鳳英編譯。1993。界面活性劑的原理與應用。高立圖書有限公司。台北。台灣。5.王建國、周忠、劉海甲、王建新。2004。甘草的活性成分及其在化妝品中的應用。日用化學工業 34(4): 249-251。
- 田慶來、官月平、張波、劉會洲。2006。甘草有效成分的藥理作用研究進展。天然產物研究與開發 18(2): 343-347。
- 白亞之。2008。微乳液的製備及其在化妝品中的應用。日用化學品科學 31(4): 26-29。
- 行政院衛生署。1990。衛署藥字第 0 九三 0 三三五三三二號公告。行政院衛生署。台北。台灣。9.行政院衛生署。2004a。化妝品衛生管理條例總則第三條。行政院衛生署。台北。台灣。10.行政院衛生署。2004b。衛署藥字第 0 九三 0 三三五三三二號公告修正。行政院衛生署。台北。台灣。11.行政院衛生署。2005。化妝品分類表。行政院衛生署。台北。台灣。12.艾德平。2008。高壓均質技術在化工行業中的應用。江西化工(4): 194-196。
- 何士慶、蘇淑茵、劉祖惠。2006。中草藥化妝保養品。科技圖書。台北。台灣。14.呂易珍。2004。液態納豆應用於化妝品之研究。靜宜大學應用化學研究所論文。台中。台灣。15.李仰川。1999。化妝品學原理。文京圖書公司。台北。台灣。16.李潔如、牟中原。1994。微胞、微乳液的形成。科學月刊 25(10): 739。
- 林仲、陳明中、陳秀錦、蘭紅光、陳訓梅。1999。急性皮膚刺激試驗評價的應用。衛生毒理學 1(13)。
- 周大鑫。2005。高壓均質技術應用簡介。機械工業雜誌 267(1): 69-78。
- 周大鑫、徐紹煜、蘇志杰、黃國饒、林定一、何立凡、張力仁。2005。高壓均質技術在健康食品與化妝保養品之應用。奈米國家型科技計畫成果發表會成果報告論文集。
- 周大鑫。2006。奈米粉體分散設備發展現況。機械工業學訊 279(1): 5-16。
- 周忠、王建國、周蕾、王建新。2003。油溶性美白劑對酪胺酸？“璠諷些鄭鳩?撗隕k。日用化學工業33(5): 326-328。
- 周東，王習魁，張裕中。2008。高壓均質過程可視化分析與探討。機械製造 46(1): 56-59。
- 柯怡如。2007。製備蝦紅素微乳液及其乳霜化妝品之探討。國立海洋大學食品科學研究所碩士論文。基隆。台灣。24.洪偉章、陳榮秀。2004。化妝品化學。高立圖書。台北。台灣。25.洪偉章、李金枝、陳榮秀。2001。化妝品原料及功能。藝軒圖書出版社。台北，台灣。26.孫宛如。2006。奈米乳液的開發與特性探討。長庚大學生化與生醫工程研究所碩士論文。桃園。台灣。27.孫芸、阿依努爾 吾買爾、燕雪花、趙虎。2009。甘草黃酮的提取方法及藥理作用研究進展。新疆中醫藥 27(1): 72-75。
- 馬淑燕、木合布力 阿布力孜、巴哈爾古麗 卡哈爾、何一倩。2007。光果甘草異黃酮類成分光甘草定的製備工藝。新疆醫科大學學報 30(7): 692-694。
- 張有義、郭蘭生編譯。1998。膠體與界面科學入門。高立書局。台北。台灣。30.張惠淇。2002。中藥美白化妝品其安全品質與療效之評估。中國醫藥學院中國藥學研究所碩士論文。台中。台灣。31.曹恆光。1998。乳液的簡介。化工 45(2): 39-52。
- 曹恆光、連大成。2001。淺談微乳液。物理雙月刊 23(4): 488-493。
- 郭俊賢、殷正華、蔣亞婷、林萃莘、郭光輝。2004。奈米生技化妝品專利地圖及分析。行政院國家科學委員會科學技術資料中心。台北。台灣。34.陳韋達譯、光井武夫著。1996a。新化妝品學。合記圖書。台北。台灣。35.陳崇賢。1996b。乳液概論。界面科學會誌 19(1): 1-11。
- 陳威丞。2004。兼具酪胺酸？“

璠謗O及抗氧化力自中藥材之篩選。大同大學生物工程研究所碩士論文。37.陳崇裕。2008。高壓均質技術於化妝品微脂粒之應用。化工資訊與商情 56:50~55。38.傅振、偉權、許滄栗。2009。噴嘴內空穴現象對柴油機噴霧特性的影響。農機化研究 31(3): 63-68。39.彭金玉、詹馥妤。2006。化妝品配方設計與實務。新文京開發出版股份有限公司。台北。台灣。40.曾淑惠。2006。界面活性劑對以溫度轉相法進行奈米乳化之影響。嘉南藥理科技大學化妝品科技研究所碩士論文。嘉義。台灣。41.楊濟華。1996。部分中草藥與美白與防曬成分研究。靜宜大學應用化學系應用化學所碩士論文。台中。台灣。42.趙坤山、劉添仁、李進興。2008。化工技術在機能性化妝保養品之應用。化工 55(3): 21-29。43.劉春敏。2004。利用酵素法提升胡蘿蔔濃縮汁收率及品質之研究。大葉大學生物產業科技研究所碩士論文。彰化。台灣。44.賴碧玉。2000。乳液安定性控制因素。元智大學化工系碩士論文。桃園。台灣。45.嚴嘉蕙。2006。化妝品概論。新文京開發出版社。台北。台灣。46.AOAC. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washing, D.C. USA. 47.Amir, M., Tayebe, T., Mohammad, R. R., Sharyar, A. and Farid, D. 2004. Design and in vitro evaluation of new drug-in-adhesive formulations of fentanyl transdermal patches. *Acta Pharmaceutica Sciencia* 54: 301-317. 48.Arriagada, F.J. and Osseo-Asare, K. 1999. Synthesis of Nanosize Silica in a Nonionic Water-in-Oil Microemulsion: Effects of the Water/Surfactant Molar Ratio and Ammonia Concentration. *Journal of Colloid and Interface Science* 211: 210-220. 49.Becher, P., 1966. Emulsions: Theory and practice. 2 th ed. Reinhold, New York. 50.Benita, S. and Levy, Mm. Y. 1993. Submicron emulsions as colloidal drug carriers for intravenous administration: Comprehensive physicochemical characterization. *Journal of Pharmaceutical Sciences* 82: 1069-1079. 51.Blois, M. S. 1958. Antioxidant determination by the use of a stable free radical. *Nature* 26: 1199-1200. 52.Bouchemal, K., Brian?帙n, S., Perrier, E. and Fessi, H. 2004. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. *International Journal of Pharmaceutics* 280: 241-251. 53.Breathnach, A. S. 1975 Aspects of epidermal ultrastructure. *J Investig Dermatol* 65: 2-15. 54.Bronaugh, R.L. and Maibach, H.I. 1989. Structure-activity Correlations in Percutaneous Absorption in: *Percutaneou Absorption: Mechanism, Methodology Drug Delivery*, 2thed, Marcel Dekker, New york, p95-109. 55.Butterworths. 1972. *International Thermodynamic Tables of the Fluid State*, Blackwells Scientific, London. 56.Chanamai, R. and McClements, D. J. 2000. Dependence of creaming and rheology of monodisperse oil-in-water emulsions on droplet size and concentration. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 172: 79-86. 57.Che, L. M., Wang, L. J., Li, D., Bhandari B., ?頃kan N., Chen, X. D and Mao, Z. H. 2009. Starch pastes thinning during high-pressure homogenization. *Carbohydrate Polymers* 75: 32-38. 58.Chen, S.H. and Lin, T.L. 1986. In *Methods of Experimental Physics-Neutron Scattering*. vol 2. NewYork . 59.Chern, C.S. and Chen, T.J. 1998. Effect of Ostwald ripening on styrene miniemulsion stabilized by reactive cosurfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 138: 65-74. 60.Chikao, N., Nobuyasu, E., Shinkichi, T., Akihisa, M., Koji, K. and Masako, F. 1987 Antibacterial Activity of Flavonoids against *Staphylococcus epidermidis*, a Skin Bacterium. *Agricultural and Biological Chemistry* 51:139-143. 61.Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid oxidat ion catalyst in muscle food. *Journal of Agricultural and Food Chemistry* 38: 674-677. 62.Del Marmol, V. and Beermann, F. 1996. Tyrosinase and related proteins in mammalian pigmentation. *FEBS Letters* 381: 165-168. 63.Delves-Broughton, J. 1990. Nisin and its application as a food preservative. *International Journal of Dairy Technology* 43: 73-76. 64.Dickinson, E., Golding, M. and Povey, M. J. W. 1997. Creaming and Flocculation of Oil-in-Water Emulsions. Containing Sodium Caseinate. *Journal of Colloid and Interface Science* 185: 515-529. 65.Duckworth, H. W. and Coleman, J. E. 1970 Physicochemical and kinetic properties of mushroom tyrosinase. *International Journal of Bioiloical Chemistry* 245: 1613-1625. 66.Ee, S. L., Duan, X., Liew, J. and Nguyen, Q. D. 2008. Droplet size and stability of nano-emulsions produced by the temperature phase inversion method. *Chemical Engineering Journal* 140: 626-631. 67.Engels, T., F?宁ster, T. and Von Rybinski, W. 1995. The influence of coemulsifier type on the stability of oil-in-water emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 99: 141-149. 68.Essawi, T. and Srour, M. 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology* 70: 343-349. 69.Fernandez, P., Andr?? V. and Rieger, J. and K?併le, A. 2004. Nano-emulsion formation by emulsion phase inversion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 251: 53-58. 70.Fessi, H., Puisieux, F., Devissaguet, J.P., Ammoury, N. and Benita, S.1989. Nanocapsule formation by interfacial polymer deposition following solvent deplacement. *International journal of pharmaceutics* 55: 25 – 28. 71.Floury, J., Desrumaux, A. and Lardi?嫫es, J. 2000. Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. *Innovative Food Science & Emerging Technologies* 1: 127-134. 72.Fuhrman, B., Volkova, N., Kaplan, M., Presser, D., Attias, J., Hayek, T and Aviram, M. 2002. Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: increased resistance of LDL to atherogenic modifications, reduced plasma lipid levels, and decreased systolic blood pressure. *Nutrition* 18: 268-273. 73.Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S. and Nomura, T. 2002. Anti-Helicobacter pylori flavonoids from licorice extract. *Life Sciences* 71: 1449-1463. 74.Gasco, M. R., Gallarate, M. and Pattarino, F. 1991. In vitro permeation of azelaic acid from viscosized microemulsions. *International Journal of Pharmaceutics* 69: 193-196. 75.Gerd, H., Dahms, Kermira, H. and Pigments, Oy. 2004. Compatibility of microfine titanium dioxide with organic UV filters. *Cosmetics and Toiletries Manufacture Worldwide*. p115-118. 76.Girard, N., Tadros, T. F. and Bailey, I. 1998. Polymerization of oil (styrene and methylmethacrylate)-in-water microemulsions. *Colloid & Polymer Science* 276: 999-1009. 77.Griffin, W.C. 1949. Classification of Surface-Active Agents by HLB. *Journal of the Society of Cosmetic Chemists* 1: 311-326. 78.Guerzoni, M.E., Suzzi, G., Lanciotti, R., Vannini, L. and Chaves Lopez, C. 1998. Effects of high pressure homogenization on biochemical and texture characteristics of yoghurt. In: *Proceedings of the IDF Symposium “ Texture of Fermented Milk Products and Dairy Desserts ”*, Vicenza, Italy, 5 – 6 May 1997, International Dairy Federation, Brussels, Belgium, pp. 172 – 180. 79.Guti?臆rez, J. M. Gonz?鳩ez, C. Maestro, A. Sol?? I. Pey, C. M. and Nolla, J. 2008. Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid & Interface Science* 13: 245-251. 80.ISO. 2008. *Cosmetics Microbiology Enumeration of yeast and mould*, International Organization for Standardization. ISO

16212:2008. Geneva, Switzerland. 81.ISO. 2006. Cosmetics Microbiology Enumeration and detection of aerobic mesophilic bacteria. International Organization for Standardization. ISO 21149:2006. Geneva, Switzerland. 82.Ivanov, I. B., Danov, K. D. and Kralchevsky, P. A. 1999. Flocculation and coalescence of micron-size emulsion droplets. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 152: 161-182.

83.Jafari, S. M., Assadpoor, E., He, Y. and Bhandari, B. 2008. Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids* 22: 1191-1202. 84.Karbstein, H. and Schubert, H. 1995. Developments in the continuous mechanical production of oil-in-water macro-emulsions. *Chemical Engineering and Processing* 34: 205-211. 85.Khatib, S., Nerya, O., Musa, R., Shmuel, M., Tamir, S. and Vaya, J. 2005. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. *Bioorganic & Medicinal Chemistry* 13: 433-441. 86.Kleinig, A. R and Middelberg, A. P. J. 1996. The correlation of cell disruption with homogenizer valve pressure gradient determined by computational fluid dynamics. *Chemical Engineering Science* 51: 5103-5110. 87.Lanciotti, R., Sinigaglia, M., Angelini, P. and Guerzoni, M.E. 1994. Effects of homogenization pressure on the survival and growth of some food spoilage and pathogenic micro-organisms. *Letters in Applied Microbiology* 18: 319-322. 88.Lanciotti, R., Gardini, F., Sinigaglia, M. and Guerzoni, M.E. 1996. Effects of growth conditions on the resistance of some pathogenic and spoilage species to high pressure homogenization. *Letters in Applied Microbiology* 22: 165 – 168. 89.Lindley, J. and Mason, T. 1987. Sonochemistry – synthetic applications. *Chemical Society Reviews* 16:275 – 311. 90.Liu, T. T., Yang, T. S., Wu, T. S., Chen, H. T., Liu, Y. X. and Yang, J. X. 2006. Study of factors affecting stability of evening primrose oil emulsion by response surface method. *Taiwanese journal of agricultural chemistry and food science* 44(2): 83-89 91.Matsuda, H., Higashino, M., Chen, W., Tosa, H., Iinuma, M. And Kobo, M. 1995. Studies of cuticle drugs from natural sources.Inhibitory effect of myrica rubra on melanin biosynthesis. *Biological pharmaceutical bulletin* 18(8):1148-50. 92.Milton, J. Rosen. 2004. *Surfactant and Interfacial Phenomena*. John Wiley & Sons ,New York. p. 1, p. 207, p. 228-229.

93.Montagna, W. and Parakkal, P. F. 1974. The structure and functiob of skin. 3th ed. New York: Academic Press. USA. 94.Napper, D.H. 1977. Steric stabilization. *Journal of Colloid and Interfacial science* 58: 390-407. 95.Olson, D.W., White, C.H. and Richter, R.L. 2004. Effect of Pressure and Fat Content on Particle Sizes in Microfluidized Milk. *Journal of Dairy Science* 87: 3217-3223. 96.Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* 44: 307-315. 97.Pandolfe, W.D., 1998. High-pressure homogenization. *Chemical processing (Chicago, I11)* 61 (3): 39 – 43. 98.Phipps, L. W. 1971. Mechanism of Oil Droplet Fragmentation in High Pressure Homogenizers. *Nature* 233: 617-619. 99.Porras, M., Solans, C., Gonz?鷇ez, C., Mart?潘ez, A., Guinart, A. and Guti?臆rez, J.M. 2004. Studies of formation of W/O nano-emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 249: 115-118. 100.Prota, G., Hu, D.-N., Vincensi, M. R., McCormick, S. A., and Napolitano, A. 1998 Characterization of Melanins in Human Irides and Cultured Uveal Melanocytes From Eyes of Different Colors, *Experimental Eye Research* 67: 293-299.

101.Rodriguez, M. E., Diz, V. E., Awruch, J., and Dicelio, L. E. 2010. Photophysics of Zinc (II) Phthalocyanine Polymer and Gel Formulation, Photochemistry and Photobiology 86: 513-519. 102.Santos-Magalh?城s, N. S., Pontes, A., Pereira, V. M. W. and Caetano, M. N. P. 2000. Colloidal carriers for benzathine penicillin G: Nanoemulsions and nanocapsules. *International Journal of Pharmaceutics* 208: 71-80.

103.Sapkota,K., Lee, E., Yang, J. H., Kwon, Y., Choi, j. and Na, Y. 2010. 4-Hydroxy-2' -Nitrodiphenyl Ether Analogues as Novel Tyrosinase Inhibitors. *Bulletin of the Korean chemical society* 31(5): 1319-1325. 104.Schmidts, T., Nocker, P., Lavi, G., Kuhlmann, J., Czermak, P. and Runkel, F. 2009. Development of an alternative, time and cost saving method of creating pseudoternary diagrams using the example of a microemulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 340: 187-192. 105.Schulman, J. H. 1943. The Theory of Emulsions and their Technical Treatment. By William Clayton. *The Journal of Physical Chemistry* 47: 294-295. 106.Schultz, S., Wagner, G., Urban, K. and Ulrich, J. 2004. High-Pressure Homogenization as a Process for Emulsion Formation. *Chemical Engineering & Technology* 27: 361-368. 107.Shanker, K., Fatima, A., Negi, A., Gupta, V., Darokar, M., Gupta, M. and Khanuja, S. 2007. RP-HPLC Method for the Quantitation of Glabridin in Yashti-madhu (Glycyrrhiza glabra). *Chromatographia* 65: 771-774. 108.Shinoda K, Kunieda H. 1983 Phase properties of emulsions: PIT and HLB in encyclopedia of emulsion technology, Vol.1, P. Becher, MarcelDekker, Inc.,New York, 337-367.

109.Solans, C., Izquierdo, P., Nolla, J., Azemar, N. and Garcia-Celma, M. J. 2005. Nano-emulsions. *Current Opinion in Colloid & Interface Science* 10: 102-110. 110.Sonneville-Aubrun, O., Simonnet, J. T. and L'Alloret F. 2004. Nanoemulsions: a new vehicle for skincare products. *Advances in Colloid and Interface Science* 108-109: 145-149. 111.Stang, M., Schuchmann, H. and Schubert, H. 2001. Emulsification in High-Pressure Homogenizers. *Engineering in Life Sciences* 1: 151-157. 112.Tadros, T., Izquierdo, P., Esquena, J. and Solans, C. 2004. Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science* 108-109: 303-318. 113.Tan, C. P. and Nakajima, M. 2005. -Carotene nanodispersions: preparation, characterization and stability evaluation. *Food Chemistry* 92: 661-671. 114.Taylor, P. 1995. Ostwald ripening in emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 99: 175-185. 115.Taylor, P. 2003. Ostwald ripening in emulsions: estimation of solution thermodynamics of the disperse phase. *Advances in Colloid and Interface Science* 106: 261-285.

116.Urbina-Villalba, G. and Garc?朦-Sucre, M. 2001. Influence of surfactant distribution on the stability of oil/water emulsions towards flocculation and coalescence. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 190: 111-116. 117.Vannini, L., Lanciotti, R., Baldi, D. and Guerzoni, M.E. 2004. Interactions between high pressure homogenization and antimicrobial activity of lysozyme and lactoperoxidase. *International Journal of Food Microbiology* 94: 123-135. 118.Venter, J. P., M?荆ler, D. G., Du Plessis, J. and Goosen, C. 2001. A comparative study of an in situ adapted diffusion cell and an in vitro Franz diffusion cell method for transdermal absorption of doxylamine, *European Journal of Pharmaceutical Sciences* 13: 169-177. 119.Walstra, P., 1996, Emulsion stability,Encyclopedia of Emulsion Technology 4: 1-66. 120.Wu, H., Ramachandran, C., Weiner, N.D. and Roessler, B.J. 2001. Topical transport of hydrophilic compounds using water-in-oil

nanoemulsions. International Journal of Pharmaceutics 220: 63-75. 121.Yasumatsu, K., Sawada, K., Moritaka, M. T., Wada, T. and Ishii, K. 1972 Whipping and emulsifying properties of soybean products. Agricultural Biology and Chemistry 36: 719-727. 122.Yokota, T., Nishio, H., Kubota, Y. and Mizoguchi, M. 1998. The Inhibitory Effect of Glabridin from Licorice Extracts on Melanogenesis and Inflammation. Pigment Cell Research 11: 355-361. 123.Yuan, Y., Gao, Y., Mao, L. and Zhao, J. 2008. Optimisation of conditions for the preparation of β -carotene nanoemulsions using response surface methodology. Food Chemistry 107: 1300-1306. 124.Yuen, S., Cheng, Ka. M. Ng. and Christianto, W. 2010. Product design: a Transdermal patch containing a traditional Chinese medicinal tincture. Industrial & Engineering Chemistry Research 49(10): 4904-4913. 125.Zhao, X., Liu, JP., Zhang, X. and Li, Y. 2006. Enhancement of transdermal delivery of theophylline using microemulsion vehicle. International Journal of Pharmaceutics 327: 58-64. 126.Zlotogorski, A. 1987. Distribution of skin surface pH on the forehead and cheek of adults. Archives of Dermatological Research 279: 398-401.