

藥學科技二報告：
Scifinder 及 Innovation 應用

主題：Diclofenac 相關合成與專利資料

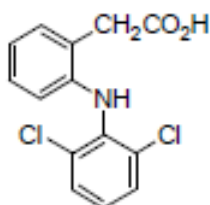
課程：藥學科技二
指導老師：吳建德老師
系級：藥學系三年級
班別：D班
學號：B303098176
姓名：洪雅庭

一、動機：

在最初兩次的藥學科技二的課程中，吳老師安排我們上機，講解並教導我們去運用 Scifinder 和 Innovation 這兩個平台來搜尋資料。Scifinder 資料廣泛，內容豐富且包含了各種面向，是十分好用的文獻搜尋資源，而 Innovation 則是便於專利的查詢。此兩個平台都是未來極有可能用得上的便利資源，而透過這次報告也讓我們有了實際練習的機會。

在藥物化學課程中，授課的劉老師介紹到了 Diclofenac 這項藥品，使我立刻想到了著名痠痛貼布廣告中的「大搵粉圓」的台詞，想說既然一直有耳聞，但是以前並不清楚這個藥到底是做什麼用的，剛好配合這次的報告，於是 diclofenac 就成為了我的報告主題。

二、藥品介紹—Diclofenac

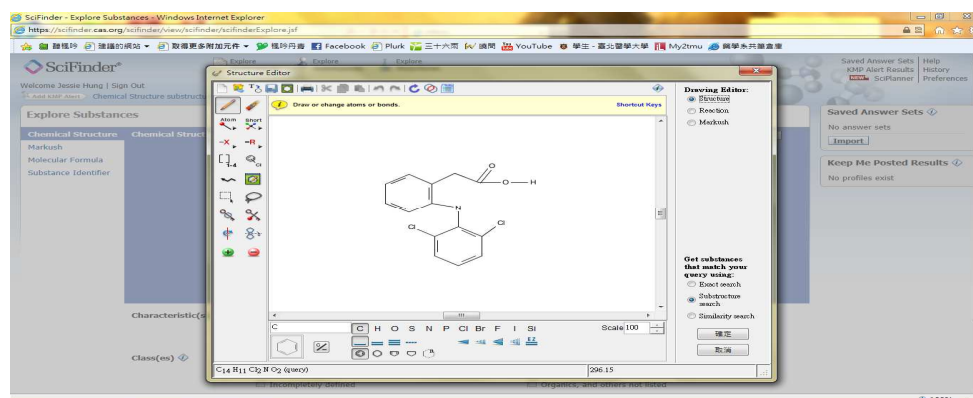


Diclofenac

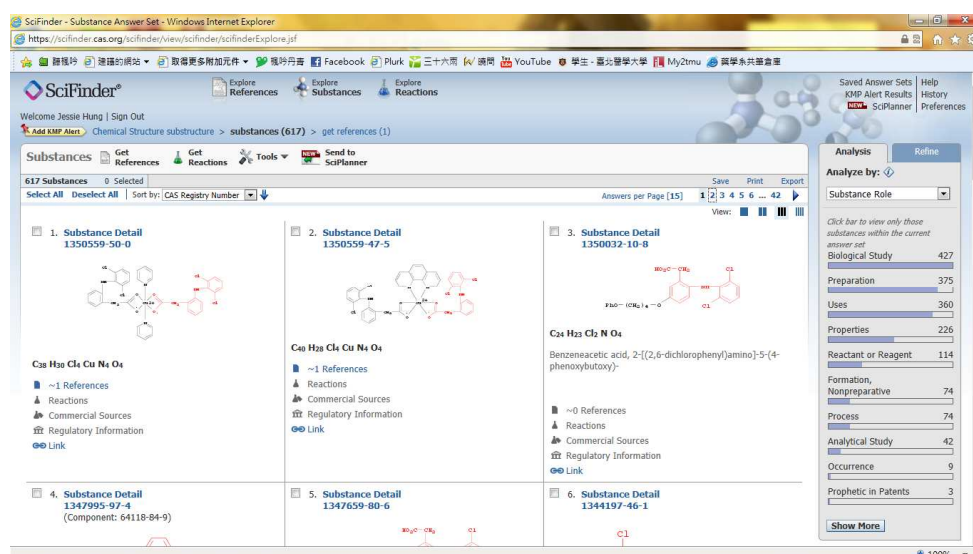
1. 為 Arylacetic acid Derivatives 的 NSAID(non-steroid anti-inflammatory drugs)
2. 為目前最廣為使用的 NSAID 類藥品
3. 作用機轉和一般的 NSAID 有所不同，十分特別：
 - (1). 抑制 arachidonic acid cyclooxygenase system
 - (2). 抑制 lipoxxygenase
 - (3). 抑制 arachidonic acid 的釋放和促進其再回收

三、Scifinder 搜尋過程

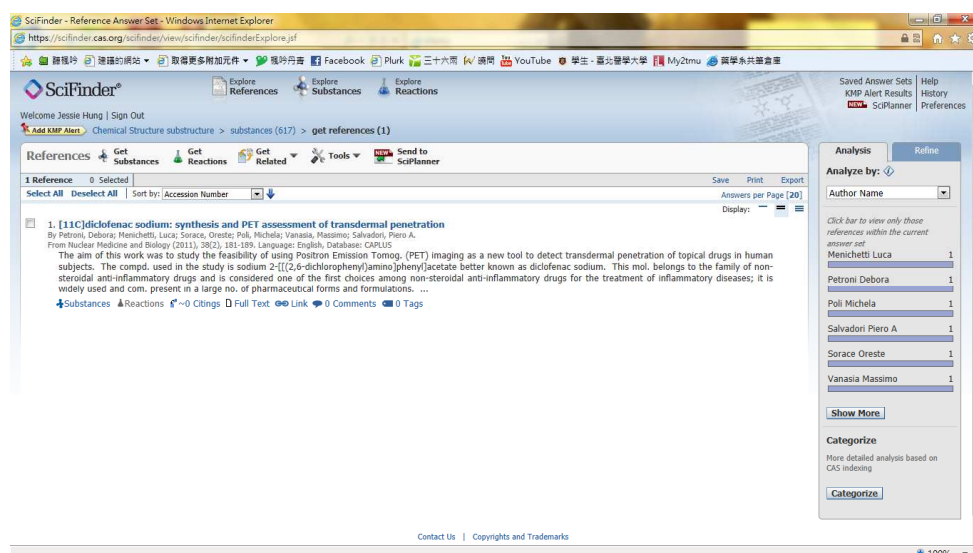
1. 一開始先在搜尋列直接打入 Diclofenac，但發現資料量過多，再鎖定近十年的時間來篩選，但仍有超過 10000 筆資料，且內容不一定符合我想要的方向，所以改用結構繪圖去找：



2. 搜尋到了六百多筆資料：



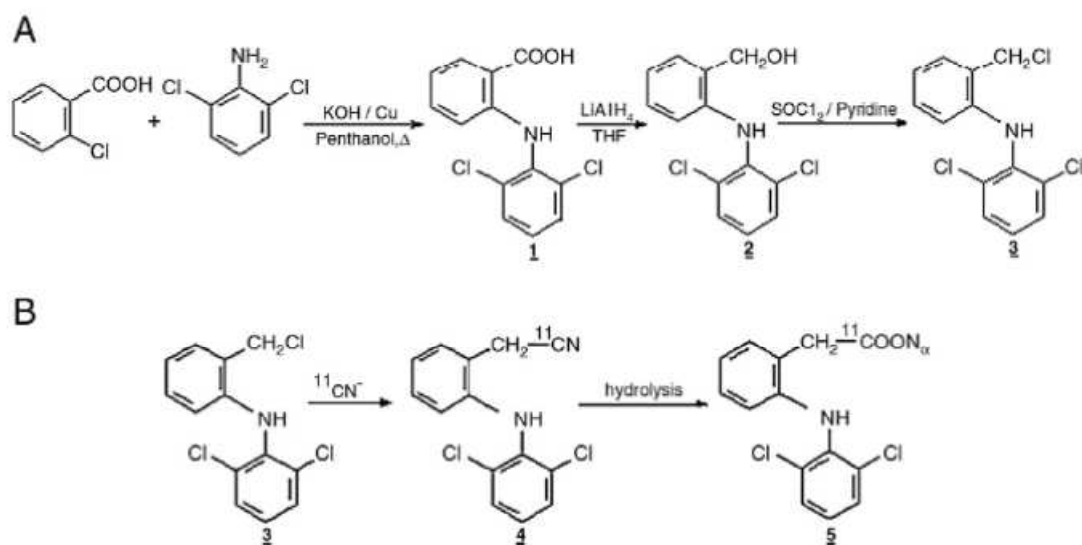
3. 點入其中一篇：



4. 載下可能會用到的數篇文獻備用。

四、合成方法

搜尋資料的過程中發現 **Dioclofenac** 不止一種合成方式，而且也有許多文獻顯示常常有研究利用 **Dioclofenac** 做結構上的修飾，研發新藥，以下節錄其中一種的合成方式：（見下圖）



Scheme 1. Synthesis of $[^{11}\text{C}]$ diclofenac sodium. (A) Synthesis of the precursor. (B) Radiochemistry.

部分文獻內容(此文獻因為研究所需，有使用 ^{11}C 做標定)：

The method is based on the synthesis of a chlorinated precursor, namely the 2-[(2,6-dichlorophenyl)amino]benzyl chloride (3), which, by reaction with sodium $[^{11}\text{C}]$ cyanide and successive hydrolysis, can be converted into $[^{11}\text{C}]$ diclofenac. The chlorinated precursor was not commercially available: it was prepared optimizing the synthesis described in the literature [3]. The sequence of reactions is reported in Scheme 1A. Reaction between 2-chlorobenzoic acid and 2,6-dichloroaniline produced 2-[(2,6-dichlorophenyl)amino] benzoic acid (1), which was then reduced with LiAlH_4 to 2-[(2,6-dichlorophenyl)amino]benzyl alcohol (2). The alcohol was chlorinated with thionyl chloride in pyridine to produce the precursor (3).

(1). Compound 1

2-Chlorobenzoic acid (52.5 g, 0.33 mol) and KOH (19.5 g, 0.35 mol) in 160 ml of 1-pentanol were heated at 100°C under stirring for 1 h. 2,6-Dichloroaniline (100 g, 0.65 mol) and copper powder (1.25 g, 0.02 mol) were then added after cooling, and the mixture was refluxed for 120 h. After cooling, a solution of Na_2CO_3 (54 g in 180 ml of water) was added to the mixture. The organic layer was separated and the aqueous phase was extracted with pentanol. Organic phases were combined and concentrated HCl was added dropwise to precipitate a brown solid. The solid was filtered under vacuum, washed repeatedly with concentrated HCl and dried under vacuum. Pure compound 1 was obtained after crystallization from EtOH/ H_2O (50/50). The yield was 21%; mp 210°C – 212°C . $^1\text{H-NMR}$: δ (CDCl_3) 6.3 (dd, 1H), 6.8 (dt, 1H), 7.1–7.5 (m, 4H), 8.1 (dd, 1H), 9.2 (s, 1H). HPLC (eluent B): retention time: 1.7 min.

(2). Compound 2

A solution of compound 1 (10 g, 35 mmol) in 80 ml of anhydrous THF was added dropwise to a suspension of LiAlH_4 in 30 ml of anhydrous THF cooled at 5°C – 15°C .

The resulting mixture was then heated and refluxed for 15 h under stirring. After cooling, 20 ml of water, 20 ml of 15% NaOH solution and 60 ml of water were carefully added at 0°C–5°C. The suspension was filtered, and the solid residue was washed on the filter with THF. The organic phase was concentrated under vacuum, and the resulting solid dissolved in ethyl acetate. The solution was washed with 2N Na₂CO₃ solution and saturated NaCl solution. The organic phase was concentrated at reduced pressure. Ethyl ether was added to the residue and pure compound **2** was recovered adding petroleum ether to the solution. The yield was 67%; mp 108°C–110°C. ¹H-NMR δ (CDCl₃): 1.8 (bs, 1H), 4.8 (d, 2H) 6.4 (dd, 1H), 6.9 (dt, 1H), 7.1–7.3 (m, 4H), 7.4 (d, 1H). HPLC (eluent B): retention time: 2.9 min.

(3). Compound 3

A solution of compound **2** (7 g, 26 mmol) in 104 ml of dry diethyl ether and 10.4 ml of anhydrous pyridine was quickly added dropwise to a solution of 10.4 ml of SOCl₂ in 56 ml of anhydrous pentane cooled at 0°C. The solution was stirred for 30 min at 0°C and then poured on ice. The mixture was extracted with 30 ml of 2N HCl and washed with 30 ml of 2N NaOH solution followed by 30 ml of water. The organic phase was filtered and dried over K₂CO₃, and the solvent evaporated at reduced pressure. Compound **3** was recovered as yellow oil and used without further purification. Yield was 47%. ¹H-NMR δ (CDCl₃): 4.6 (d, 2H) 6.4 (dd, 1H), 6.9 (dt, 1H), 7.1–7.3 (m, 4H), 7.4 (dd, 1H). HPLC (eluent A): retention time: 8.8 min. Direct HPLC-MS identification of compound **3** was attempted with a triple quadrupole (Applied Biosystems Sciex API 4000) mass spectrometer coupled with a Perkin Elmer Series 200 Micro HPLC system using MeOH as eluent. Under these conditions, we observed a quantitative conversion of the chloro derivative into the corresponding methoxy derivative (molecular peaks at m/z=282 and m/z=284).

(4). 2-[(2,6-Dichlorophenyl)amino]phenyl]acetonitrile

Potassium cyanide (100 μ g, 1.5 μ mol) was added to a solution of 10 mg (33 μ mol) of compound **3** in 500 μ l of DMSO in a capped V-shaped vial. The mixture was heated at 40°C for 20 min. The solution was used in HPLC (eluent A) without purification of the product as comparison for compound **4**. Retention time: 11.4 min; purity: 92%.

(5). Sodium [¹¹C]cyanide

[¹¹C]CO₂ was produced at the cyclotron and trapped into a stainless steel loop cooled with liquid nitrogen. At the end of the delivery, the cooling bath was removed and [¹¹C]CO₂ was swept by a nitrogen stream (60 ml/min), mixed on-line with pure hydrogen (25 ml/min), and the mixture passed through an oven containing nickel kieselguhr heated at 485°C to achieve reduction to [¹¹C]methane. [¹¹C]methane was purified on-line passing through a trap of magnesium perchlorate and one of ascarite before being mixed with ammonia gas (15 ml/min) and converted to H₁₁CN in a furnace containing platinum wool

heated at 1000°C. H[¹¹C] CN was recovered by bubbling in a V-shaped vial closed by a screw cap and a silicon septum containing 500 µl of absolute ethanol, 25 µl of 1 M NaOH and 25 µl of 1.5 M KCN. The solvent was evaporated under nitrogen flow, and the resulting sodium [¹¹C]cyanide solution was used without purification.

(6). 1-[¹¹C]-2-{2-[(2,6-Dichlorophenyl)amino]phenyl}} acetonitrile (4)

Thirty milligrams (0.1 mmol) of compound **3** in 1.5 ml of DMSO was added to the vial containing Na¹¹CN (2.5 mCi). The solution was heated at 110°C for 10 min under stirring. ¹¹C-labeled compound **4** was not isolated. Radio- HPLC (eluent A) retention time: 11.3 min; radiochemical purity: 84%.

(7). [¹¹C]diclofenac sodium (5)

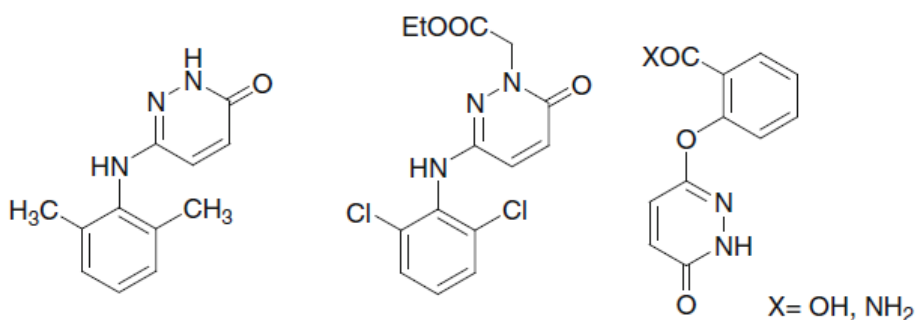
The vial containing crude ¹¹C-labeled compound **4** was added with 10N NaOH and 0.5 ml of 30% H₂O₂, and the mixture was heated at 135°C for 10 min under stirring. ¹¹C-labeled compound **5** was identified on the basis of the HPLC features of the unlabeled compound and was obtained (N°=20) with a radiochemical yield of 43%±3.8. HPLC (eluent A): 7.9 min. Pure ¹¹C-labeled compound **5** was obtained by HPLC in the same condition. Radiochemical purity: 97%±4.2 (N°=20). No chemical impurities were detected (detection limit for cold diclofenac was 3 µg/ml UV/VIS at 254 nm)

(8). Final formulation

The formulation to be used for administration consisted of a non-pressurized sprayer. The same components of the sprayer (vial, seal and cap containing the piston) were received from the manufacturer. Before each experimental session, the amount of [¹¹C]diclofenac radioactivity (180±20 MBq, 100–200 µl according to radioactive concentration) was withdrawn from the bulk, placed into the sprayer vial and dried under gentle nitrogen stream for 1 min. The capping system was removed from a diclofenac sprayer formulation, and 3 ml of the preparation was withdrawn and transferred to the vial with the radioactivity. The vial was closed again with the capping system, shaken and placed into a shielded container.

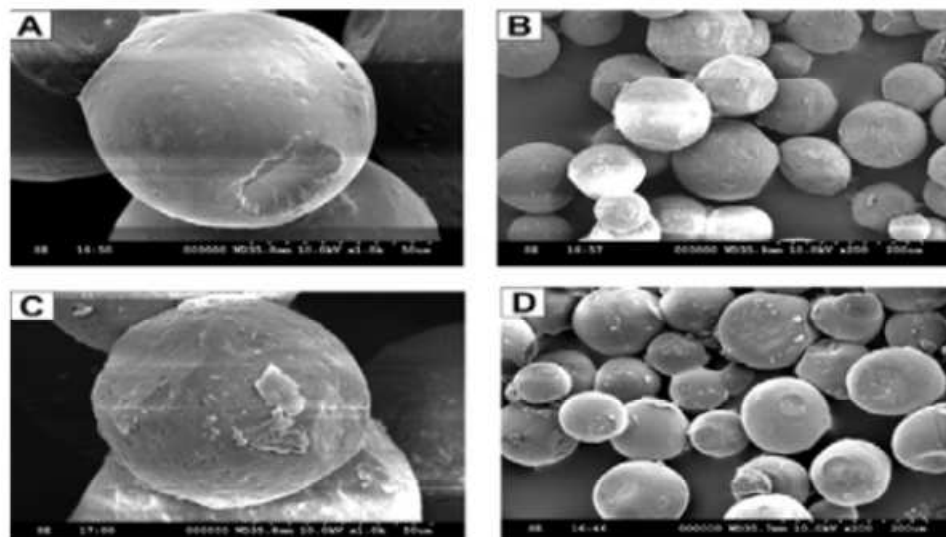
五、其他文獻：

1. 有利用diclofenac做結構修飾者：



參考文獻：K. A. M. Abouzid • N. A. Khalil • E. M. Ahmed • A. Esmat • A. M. Al-Abd (2011) Design, synthesis, and evaluation of anti-inflammatory and ulcerogenicity of novel pyridazinone derivatives.

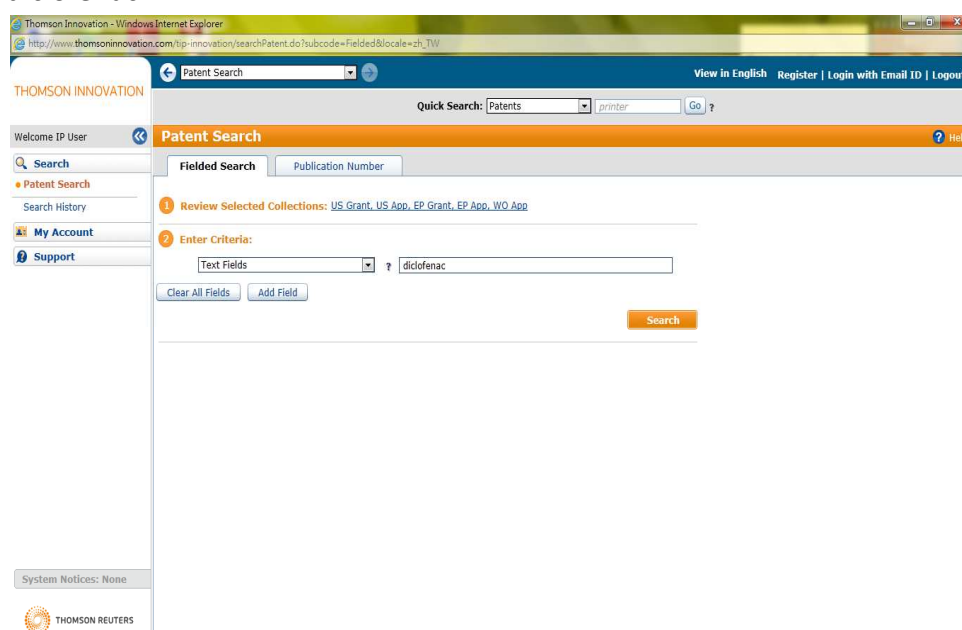
2. 設計microsphere劑型改良diclofenac：



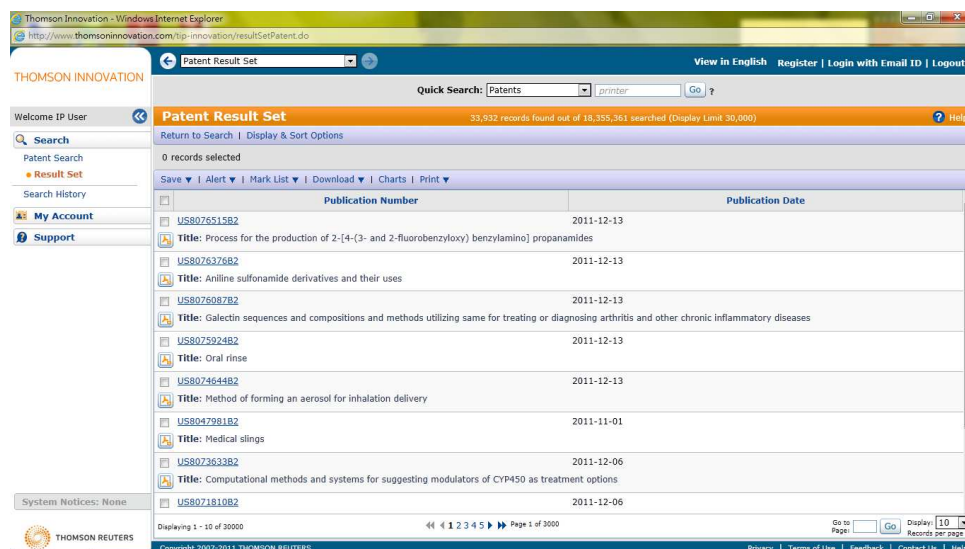
參考文獻：G.DINESH BABU*, B.VISHNU VARDHAN REDDY, S.RAJEEV CHANDRA, A.SUBHASH CHANDRA, VINOD KUMAR KH.(2011) Preparation and evaluation of ethyl cellulose and eudragit based microspheres of diclofenac potassium using double emulsion-solvent evaporation method.

六、Innovation 搜尋過程：

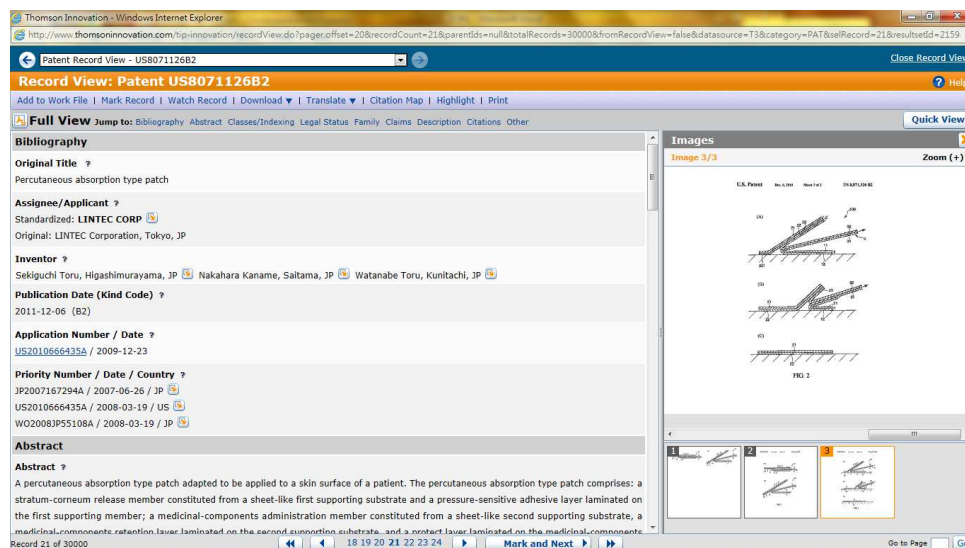
1. 透過學校研發處專利搜索平台進入 Innovation 頁面，在搜尋列輸入 diclofenac：



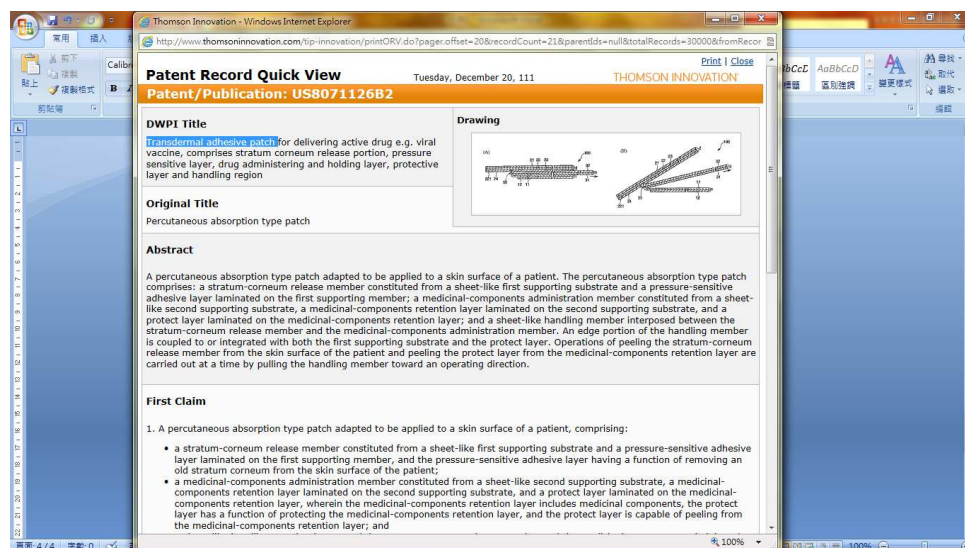
2. 搜尋到了三萬多筆資料，過多：



3. 增加搜尋條件「發表時間 2006-2011」後，點入感興趣的一篇：



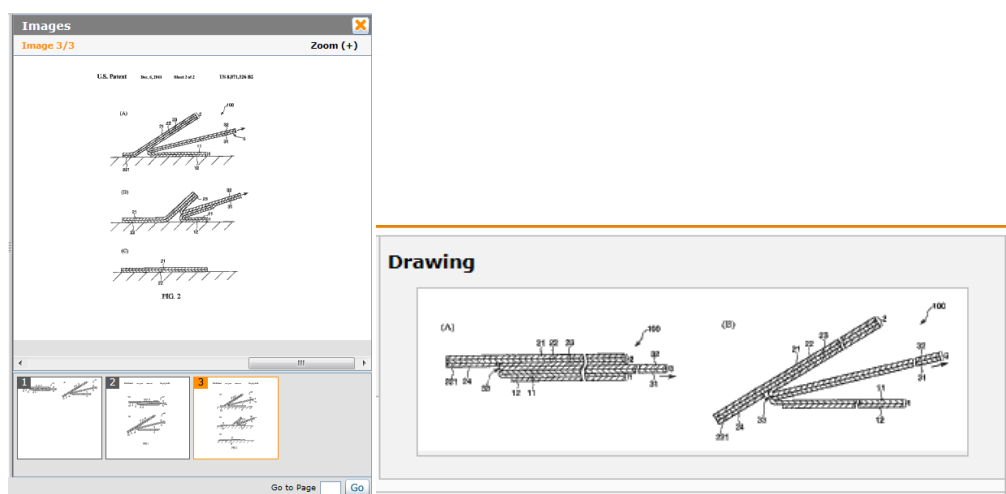
4. 專利全文下載：



七、Diclofenac 相關專利：

關於 diclofenac 的專利文相當的多，且由於我鎖定的年分比較近，所以相關專利較少是在說合成 diclofenac 的方式，大多數的內容是 diclofenac 相關衍生，例如原本是 diclofenac sodium，而改成 diclofenac potassium；而關於新劑型的設計的專利文也有不少，考慮到一開始的動機和肌力的廣告有關，所以以下挑了一篇關於 diclofenac **transdermal adhesive patch** 的專利：

1. 此項專利乃是由日本東京 LINTEC 公司所研發申請。
2. 2009 年開始申請，至 2011 通過
3. 主要是設計關於 diclofenac patch 中藥物的控釋



節錄部分專利內容：

1. DWPI Title

Transdermal adhesive patch for delivering active drug e.g. viral vaccine, comprises stratum corneum release portion, pressure sensitive layer, drug administering and holding layer, protective layer and handling region

2. Original Title

Percutaneous absorption type patch

3. Abstract

A percutaneous absorption type patch adapted to be applied to a skin surface of a patient. The percutaneous absorption type patch comprises: a stratum-corneum release member constituted from a sheet-like first supporting substrate and a pressure-sensitive adhesive layer laminated on the first supporting member; a medicinal-components administration member constituted from a sheetlike second supporting substrate, a medicinal-components retention layer laminated on the second supporting substrate, and a protect layer laminated on the medicinal-components retention layer; and a sheet-like handling member interposed between the stratum-corneum release member and the medicinal-components

administration member. An edge portion of the handling member is coupled to or integrated with both the first supporting substrate and the protect layer. Operations of peeling the stratum-corneum release member from the skin surface of the patient and peeling the protect layer from the medicinal-components retention layer are carried out at a time by pulling the handling member toward an operating direction.

八、參考資料：

1. K.P.R. Chowdary* and B.L.R. Madhavi (2011) PREPARATION AND EVALUATION OF HPMC-ALGINATE MUCOADHESIVE MICROCAPSULES OF DICLOFENAC FOR CONTROLLED RELEASE
2. David R Lionberger and Michael J Brennan (2010) Topical nonsteroidal anti-inflammatory drugs for the treatment of pain due to soft tissue injury: diclofenac epolamine topical patch
3. Debora Petronia,, Luca Menichettia, Oreste Soracea (2011) [11C]diclofenac sodium: synthesis and PET assessment of transdermal penetration
4. G.DINESH BABU*, B.VISHNU VARDHAN REDDY, S.RAJEEV CHANDRA, A.SUBHASH CHANDRA, VINOD KUMAR KH.(2011) Preparation and evaluation of ethyl cellulose and eudragit based microspheres of diclofenac potassium using double emulsion-solvent evaporation method.
5. K. A. M. Abouzid • N. A. Khalil • E. M. Ahmed •A. Esmat • A. M. Al-Abd (2011) Design, synthesis, and evaluation of anti-inflammatory and ulcerogenicity of novel pyridazinone derivatives.
6. Chao-meng Daia,b, Xue-fei Zhou**b**, Ya-lei Zhang**b**, , Shu-guang Liua, Juan Zhang**b** (2011) Synthesis by precipitation polymerization of molecularly imprinted polymer for the selective extraction of diclofenac from water samples
7. Jacques Gosteli, Basel (CH) (2006) Quaternary 3-Amido, N-Methylpyridinium salts as anti-inflammatory agents.

九、學習心得：

以前在搜尋研究文獻的時候，大多想到的都是利用 Pubmed 去尋找，但找久了有發現 Pudmed 的資料並非全面性的，感覺比較偏重在生化方面，而正好這次的藥學科技，就介紹了 Scifinder 和 Innovation 這兩個方便的平台，大概最麻煩的就是 Scifinder 有限制登入人數這點了吧！而且一開始還把當初設定的帳號密碼通通忘光光了啊！（當下內心真的有點欲哭無淚。）

在找資料的過程中，真的深刻體認到研究領域的廣闊，光是一樣藥品輸入進去，出來的結果有幾萬筆，這個驚人的數字一開始讓我愣了一會，想說這下到底要怎麼樣篩選資料呢？幸好還記得上課時，專員講解的時候有介紹怎麼樣輸入各種條件去縮小搜尋範圍，等到範圍變小而且真正找到自己要用到的資料的時候真的很開心。

一般上課幾乎都是老師給予，學生聽講然後去記憶課程內容，而這次做報告大概就是所謂「教我們釣魚的方法而不是給我們魚吃」。主題自訂本來一開始覺得很麻煩，但是後來在正課中提到靈感，然後自己設定完成這份報告的時的成就感難以形容，是做一般實驗結報無可比擬的。也透過這次實作報告，對於正課內容有了多一點的了解和認識。而且為了這次的報告，在過程中一次看了十幾篇的原文 paper，頓時覺得自己不但英文閱讀能力有提升而且似乎更有身為大學生的專業感，而不像以前搜尋資料用 google／yahoo／wiki 等。

雖然這份報告真的是頗麻煩，事前準備的功夫要一堆才能完成這樣一分作業，但是感覺自己能力有所提升，就覺得很值得。