

THE HONG KONG  
POLYTECHNIC UNIVERSITY

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香港理工大學

**Enhancing Learning and Teaching of Chemistry:**

## *Experiments on Contemporary Chemistry Topics*

### MANUAL

General Information

Experiments:

- ◆ Solventless Aldol Reaction
- ◆ Preparation and Optical Properties of CdSe Quantum Dots Nanocrystals
- ◆ Chemical Analysis of Gancuo Raw Herbs and Commercial Products

July 2006

DEPARTMENT OF APPLIED BIOLOGY & CHEMICAL TECHNOLOGY

**Enhancing Learning and Teaching of Chemistry:**

***Experiments on Contemporary Chemistry Topics***

**Programme**

|                  | Group I   | Group II  | Venue    |
|------------------|---|---|----------|
|                  |   |   |          |
| 8:45 – 9:20 am   | Registration  |   | TU103    |
| 9:20 – 10:00     | Briefing  |   | TU103    |
| 10:00 – 11:20 am | Solventless Aldol Reaction                                    | Preparation and Optical Properties of CdSe Nanocrystals | Y1315    |
| 11:20 – 12:40 pm | Preparation and Optical Properties of CdSe Nanocrystals       | Solventless Aldol Reaction                              | Y1315    |
| 12:40 – 2:00 pm  | Lunch   |   |          |
| 2:00 – 2:20 pm   | Briefing  |   | TU103    |
| 2:20 – 2:30 pm   | Group Photo   |   | Y podium |
| 2:30 – 4:00 pm   | Chemical Analysis of Gancan Raw Herbs and Commercial Products |   | Y1315    |
| 4:00 – 5:00pm    | Questions and Evaluations                                     |   | TU103    |

**Helpdesk**

Please contact Ms. Sally Yu at 9121 2755 and Mr. Bryan Fung at 9726 3362 for assistance.

URL: <http://www.polyu.edu.hk/abct/CTviva/tcorner>

# 校園地圖 Campus Map

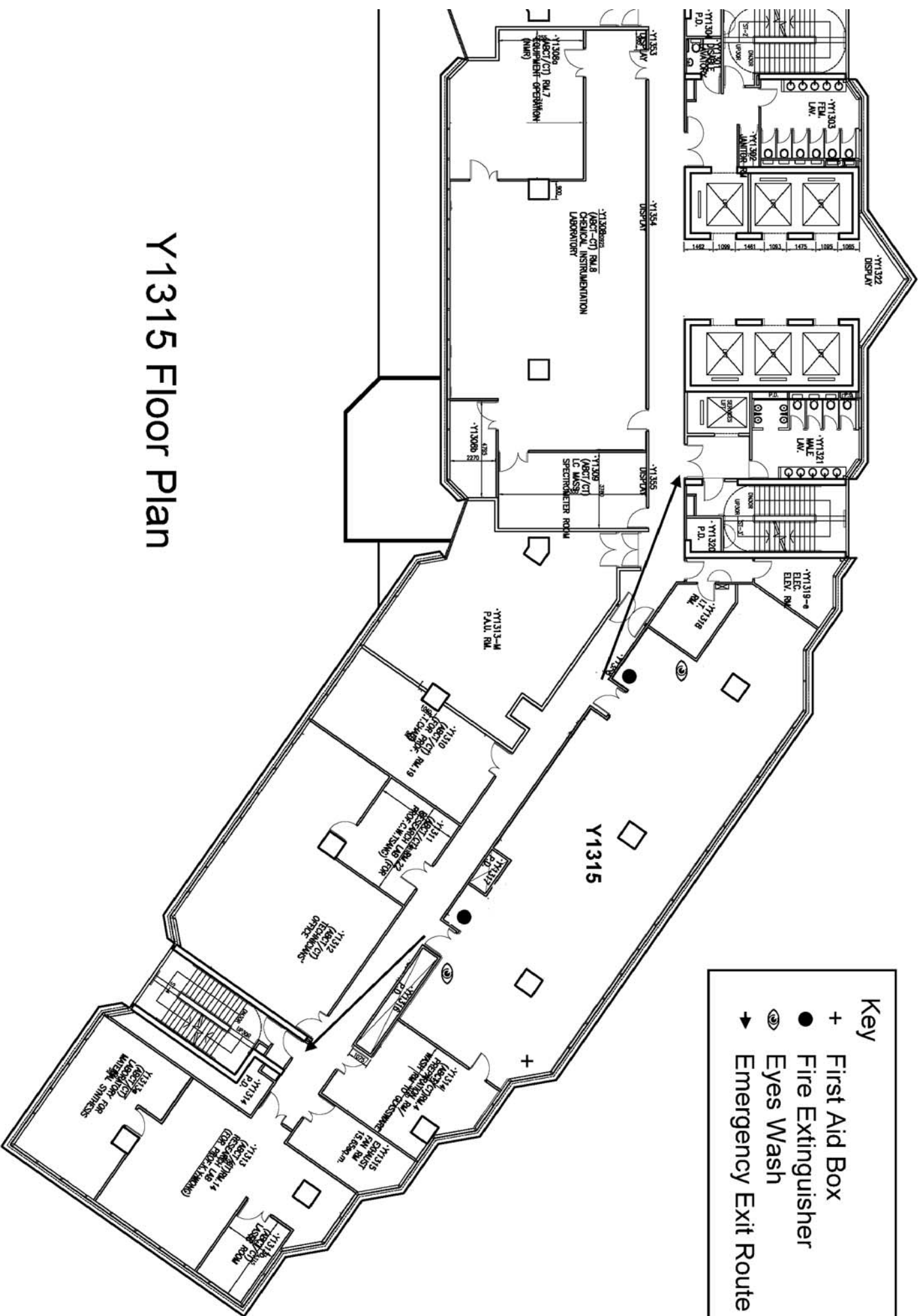
Helpdesk:  
Ms. Sally Yu 9121 2755  
Mr. Bryan Fung 9726 3362



## KEY TO CAMPUS MAP 校園索引

- |  |   |  |                      |
|--|---|--|----------------------|
|  | 建築物/設施編號                                  |  | 洗手間                  |
|  | Building / Facilities Reference Number    |  | Toilet               |
|  | Wing of Building                          |  | 殘障人士洗手間              |
|  | 物業管理處 校園保安控制中心                            |  | Canteen / Restaurant |
|  | FMO Campus Security Control Centre (P111) |  | 便利店                  |
|  | 大學醫療保健處                                   |  |                      |
|  | University Health Service                 |  |                      |





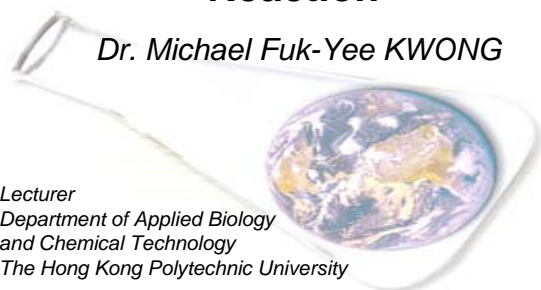


THE HONG KONG POLYTECHNIC UNIVERSITY  
DEPARTMENT OF APPLIED BIOLOGY &  
CHEMICAL TECHNOLOGY

## Solventless Aldol Reaction

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Lecturer  
Department of Applied Biology  
and Chemical Technology  
The Hong Kong Polytechnic University



## Outline:

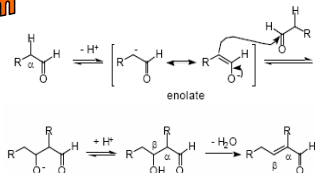
1. What is Aldol Reaction?
2. Concept of Green Chemistry
  - ~Solventless Reaction
  - ~Atom Economy
  - ~Environmental Friendly Solvent
3. Experimental Section



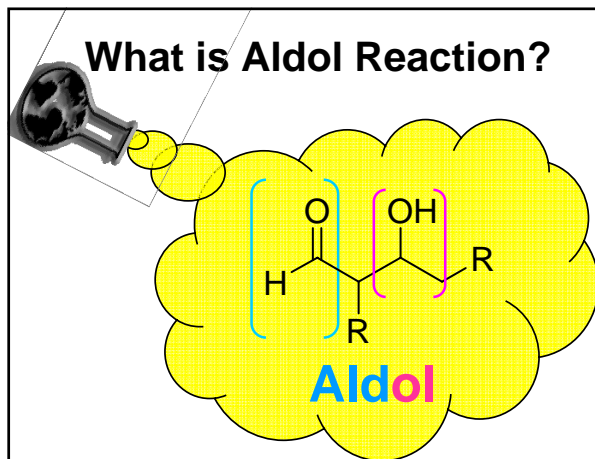
## What is Aldol Reaction?

A powerful general method for the construction of **carbon-carbon bonds**, one of the central themes of synthetic organic chemistry.

### Mechanism

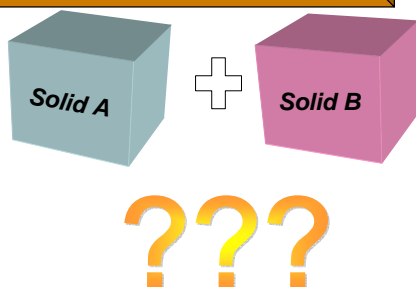


## What is Aldol Reaction?



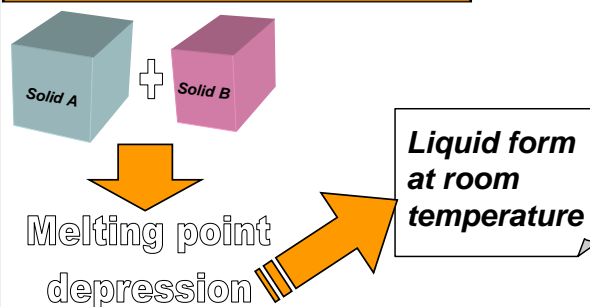
## Concept of Green Chemistry

**Solventless reaction.....**



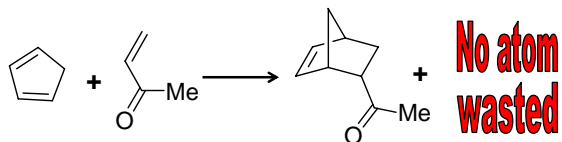
## Concept of Green Chemistry

**Solventless reaction.....**



## Concept of Green Chemistry

### Atom Economy.....



- Design syntheses so that the final product contains the maximum proportion of the starting materials (ATOM EFFICIENT).
- There should be few, if any, wasted atoms.

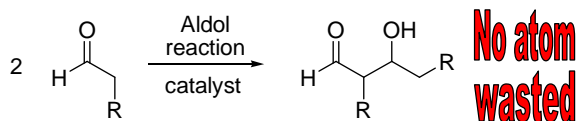
## Concept of Green Chemistry

### Atom Economy.....

- Design syntheses to use and generate substances with little or no toxicity to humans and the environment.

## Concept of Green Chemistry

### Atom Economy.....



~100% Atom efficient

## Concept of Green Chemistry

### Environmental Friendly Solvents.....

#### Examples

Water.....  
Alcohol.....  
Others???

## Concept of Green Chemistry

### Environmental Friendly Solvents.....

#### Examples

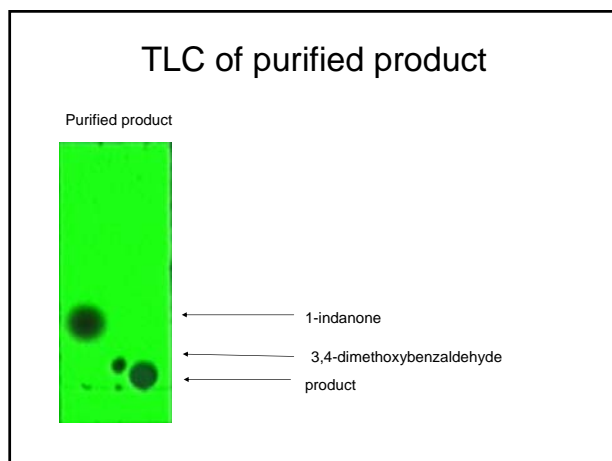
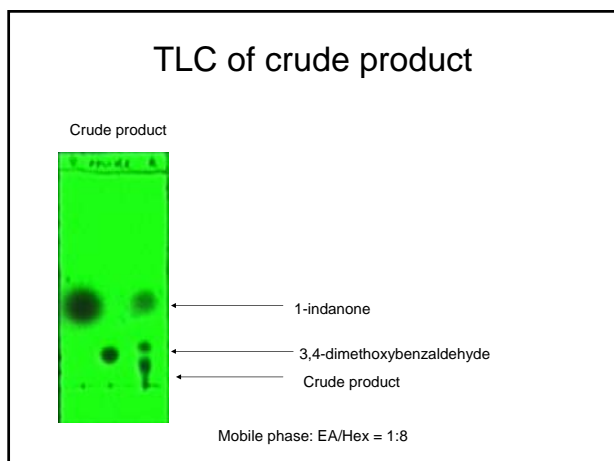
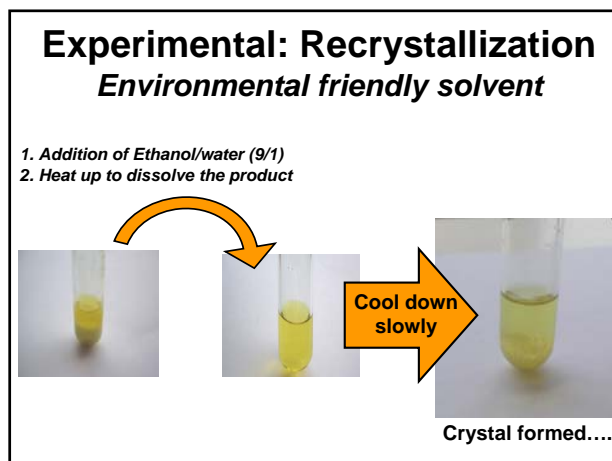
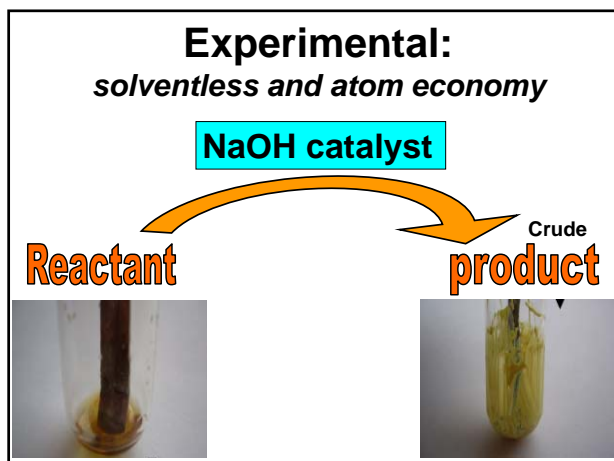
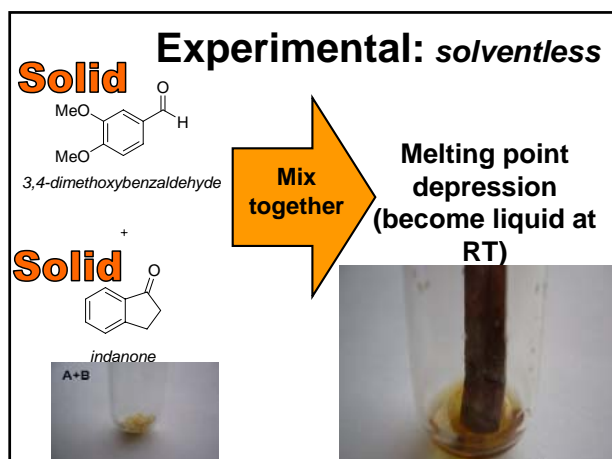
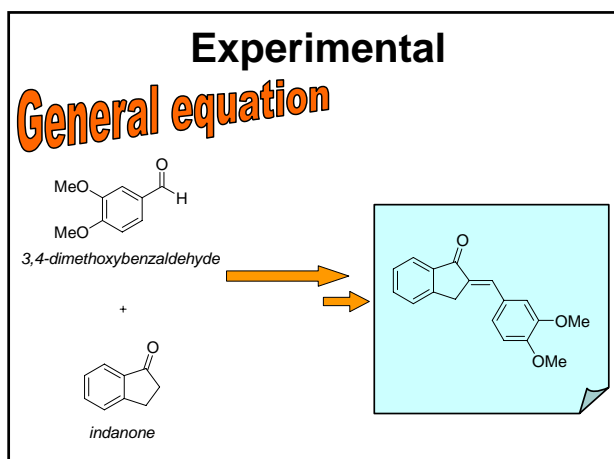
Carbon dioxide  
 $\text{CO}_2$

## Concept of Green Chemistry

### Environmental Friendly Solvents.....

- Liquid  $\text{CO}_2$  can be used as a safer solvent if a wetting agent is used with it to dissolve grease.
- This method is now being used commercially by some dry cleaners.





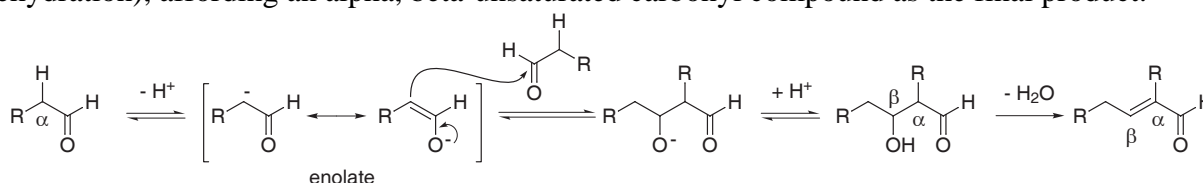
## Solventless Aldol Reaction

### Objective

To carry out aldol condensation in a solventless environment.

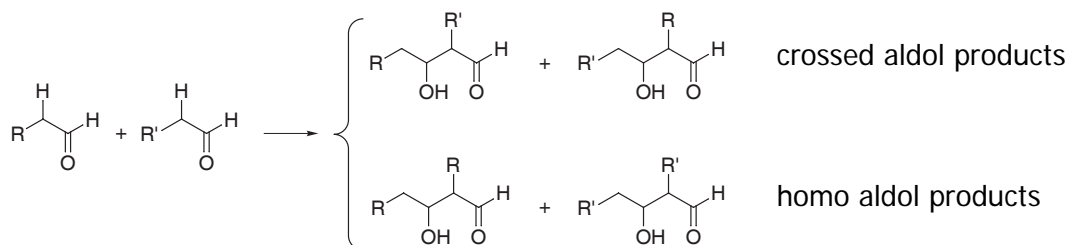
### Introduction

The aldol condensation represents a powerful general method for the construction of carbon-carbon bonds, one of the central themes of synthetic organic chemistry. In the base-catalyzed aldol condensation reaction, deprotonation  $\alpha$  (adjacent) to a carbonyl group affords a resonance-stabilized anion called an enolate, which then carries out nucleophilic attack at the carbonyl group of another molecule of the reactant. (Analogous acid-catalyzed reactions are also well-known.) The product, a  $\beta$ -hydroxy carbonyl compound, often undergoes facile elimination of water (dehydration), affording an  $\alpha, \beta$ -unsaturated carbonyl compound as the final product.



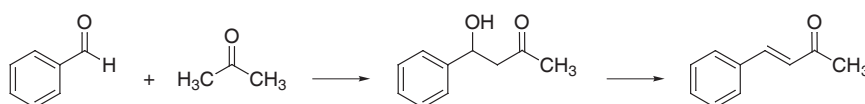
#### *Mechanism of the base-catalyzed aldol condensation*

Aldol condensation reactions between two different carbonyl compounds can lead to complex product mixtures, due to the possibility of enolate formation from either reactant and to the possibility of competing “homo” coupling rather than the desired “cross” coupling.



#### *“Crossed” aldol condensation can afford complex mixtures*

If, however, only one of the carbonyl compounds has  $\alpha$  hydrogens available for deprotonation and enolate formation, the “crossed” aldol reaction can provide synthetically useful yields of products. Thus, for example, benzaldehyde cannot be converted to an enolate, yet reacts readily with enolates of other carbonyl compounds, including acetone.



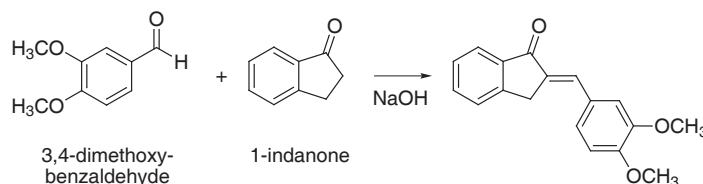
#### *A successful “crossed” aldol condensation*

Homo coupling of acetone (or other ketones) is generally not a problem in such reactions, as the aldol condensation of ketones is generally not a very efficient reaction. (More specifically, each step of the aldol condensation is reversible under the reaction conditions – at least until the dehydration



step – and thus equilibrium is established. With aldehydes, equilibrium favors the aldol product, but with ketones, primarily for steric reasons, very little aldol condensation product is present at equilibrium.)

In this experiment, you will explore the aldol condensation reaction of 3,4-dimethoxybenzaldehyde and 1-indanone.



In contrast to typical experimental procedures for aldol condensation reactions, this reaction will be carried out without solvent. Ongoing research is revealing a number of reactions that proceed nicely in the absence of solvent, representing the best possible solution to choice of a benign solvent. Although these reactions are frequently referred to as “solid-state” reactions, it has been noted [1] that in many cases, mixture of the solid reactants results in melting, so that the reactions actually occur in the liquid, albeit solvent-free state. This melting phenomenon is interesting and actually represents one of the key points of this experiment. You have learned that impurities lead to lower melting points. Here, you will experience this in a vivid way – as you mix the two solid reactants, they will melt. In addition to providing a memorable demonstration of the impact of impurities on melting points and illustrating the possibility of carrying out organic reactions in the absence of solvents, this experiment highlights another key green concept – the design of efficient, atom-economical reactions. The aldol condensation, if effected without dehydration, has an atom economy of 100% and requires only a catalytic amount of acid or base, and even with dehydration, the atom economy remains quite high.

## Experimental Procedure

### Materials

3,4-dimethoxybenzaldehyde, 1-indanone, Sodium hydroxide (pellet or powder), Hydrochloric acid, 90% Ethanol, Ethyl acetate, Hexane

### Hazards

3,4-dimethoxybenzaldehyde and sodium hydroxide are very hazardous in case of eye contact, of skin contact, of ingestion, of inhalation. Eye contact with sodium hydroxide can result in corneal damage or blindness; severe over-exposure can produce lung damage, choking, unconsciousness or death. Hydrochloric acid is very hazardous in case of eye contact, of skin contact, of ingestion; slightly hazardous in case of inhalation. Sodium hydroxide and hydrochloric acid are highly corrosive as well. 90% ethanol, ethyl acetate, and hexane are flammable. 90% ethanol is slightly hazardous in case of skin contact, of eye contact. Ethyl acetate and hexane are hazardous in case of ingestion, of inhalation, of skin contact, of eye contact. Ethyl acetate is toxic to mucous membranes, upper respiratory tract as well.

**SAFETY PRECAUTIONS:** Use care to avoid contact with solid sodium hydroxide or the reaction mixture.

### **Reaction**

1. Place 0.25g of 3,4-dimethoxybenzaldehyde and 0.20g of 1-indanone in a test tube. Using a metal spatula, scrape and crush the two solids together until they become a brown oil. Make sure no solid reactant left inside the test tube.  
(**N.B. Take special care to avoid breaking the test tube**)
2. Add about 0.05g of finely ground solid NaOH to the mixture and continue scraping until the mixture become yellowish brown solid.

### **Workup and purification**

1. Add about 2ml 10% aqueous HCl solution to the solid. Scrape well in order to dislodge the solid from the walls of the test tube. Check the pH of the solution to make sure it is acidic.
2. Isolate the solid product by vacuum filtration. (**You may determine the mass of the crude product before the recrystallization process.**)
3. Put the crude product in another test tube and add minimum amount of 90% ethanol for recrystallization. (**Hints: You may try 2ml ethanol for every 0.1g solid.**) Heat the mixture in the steam bath until all solid dissolve. After a period of time, white needle shape crystal formed and the recrystallized product can be filtered by vacuum filtration. (**Hints: You may use ice bath for a faster recrystallization step.**)

### **Characterization**

1. Determine the mass and melting point of the recrystallized product. (A typical melting point range is 178 – 181 °C.)
2. You may also check your product by TLC method. Dissolve the reactants and the recrystallized product by ethyl acetate. Use ethyl acetate/hexane mixture (1:8) as developing solvent. Watch the spots under the UV lamp box.

### **Post-Lab Questions and Exercises**

1. Describe the physical properties (color and state) of your crude product. Report the mass and percent of theoretical yield of the crude product.
2. Report the color and melting point range of your recrystallized product. Report the mass and percent of theoretical yield of the recrystallized product.
3. Calculate the atom economy for the reaction.

### **Acknowledgment:**

This experiment was provided by the Green Chemistry Institute of the American Chemical Society. We gratefully acknowledge their support.

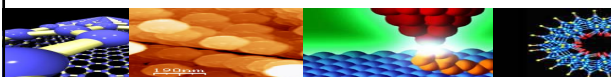
### **Reference**

1. G. Rothenberg, A. P. Downie, C. L. Raston, and J. L. Scott, "Understanding Solid/Solid Organic Reactions," *J. Am. Chem. Soc.* **2001**, 123, 8701-8708.

# Preparation and Optical Properties of CdSe Quantum Dots Nanocrystals

Dr. Michael W.-Y. Yu

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Department of Applied Biology and Chemical Technology  
The Hong Kong Polytechnic University



## Outline

- What is "Quantum Dots (QD)"?
- Chemistry of CdSe and semiconductor properties
- Band Theory
- Preparation of monodisperse CdSe nanocrystals
- Experimental and Results

## What is "Quantum Dots"?

- CdSe quantum dots are *nanocrystals* that form a colloidal solution
- These nanocrystals have a size range from 2- 15 nanometer (nm)

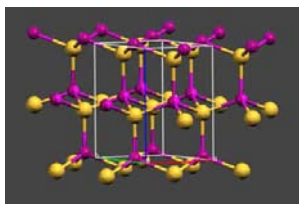


High resolution transmission electron micrograph of CdSe  
(adapted from [www.people.man.ac.uk/~mbdsspo2/nanodis.html](http://www.people.man.ac.uk/~mbdsspo2/nanodis.html))

## Chemistry

- Cd:  $[\text{Kr}]4d^{10}5s^2$ , Gp IIB (*isoelectronic with Zn*)  $\Rightarrow$  formation of dication ( $\text{Cd}^{2+}$ )
- Se:  $[\text{Ar}]3d^{10}4s^24p^4$ , Gp VI (*isoelectronic with O, S*)  $\Rightarrow$  formation of dianion ( $\text{Se}^{2-}$ )
- CdSe, *salt-like* compound
- Cd chalcogenides (e.g. CdS) are used as industrial color pigments

## Crystal Structure



Adapted from [www.york.ac.uk/.../mx.htm](http://www.york.ac.uk/.../mx.htm)

- ZnS (wurtzite)  $\Rightarrow$  each Cd / Se bonded tetrahedrally to four other counterions
- *Diamond-like* structure  $\Rightarrow$  isostructural to Si

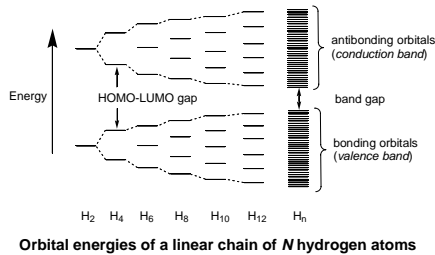
## Electrical Properties of Materials

- **Electrical conductivities:**
  - Cu, Ag (conductor):  $10^6$  S/cm
  - Ge, Si (semiconductor):  $10^{-2}$  -  $10^{-5}$  S/cm,
  - Glass, diamond, teflon (insulator):  $10^{-10}$  -  $10^{-18}$  S/cm
- **Temperature effect:**
  - For metal: conductivity  $\downarrow$  when  $T \uparrow$
  - For semiconductors: conductivity  $\uparrow$  when  $T \uparrow$

## Band Theory

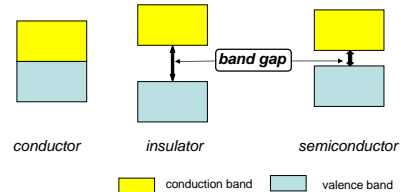
- Interaction of atomic orbitals in solid leads to numerous molecular orbitals that are closely spaced in energy and so became a continuous band of energy level

- HOMO = Highest Occupied Molecular Orbital;
- LUMO = Lowest Unoccupied Molecular Orbital



## Band Gap and Electrical Conductivity

- Metal (Cu, Ag):** No band gap  $\Rightarrow$  a continuum of filled and empty electronic states  $\Rightarrow$  electronic excitation occurs at all temp  $> 0\text{ K} \Rightarrow$  conduction band well populated  $\Rightarrow$  high electrical conductivity
- Non-metal (diamond):** Large band gap due to strong covalent bonds  $\Rightarrow$  conduction band largely unpopulated  $\Rightarrow$  electrical insulator
- Semiconductor (Si, Ge):** Small band gap  $\Rightarrow$  all electrons remain in the valence band at  $0\text{ K} \Rightarrow$  thermal excitation at temp  $> 0\text{ K}$  increases electron density in the conduction band  $\Rightarrow$  electrical conductivity rises with higher temperature

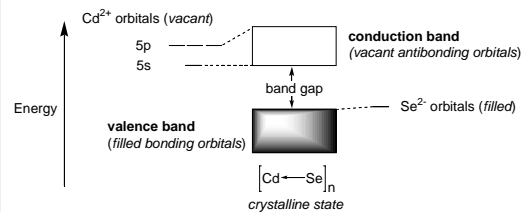


## Electrical Properties of CdSe

- Cd good electrical conductor;
- Se electrical insulator
- CdSe is a *semiconductor* that conducts electricity upon external stimuli (e.g. heat, light, voltage)
- Why?

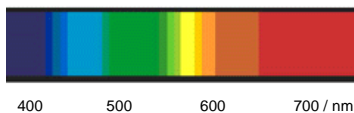
## Band Theory Description of CdSe

- In pure silicon, Si atoms are connected by homonuclear covalent bonds
- In CdSe, the Cd-Se linkage is considered as coordinative bond, i.e.,  $\text{Se}^{2-}$  (ligand) datively bonding to  $\text{Cd}^{2+}$  ion



Simplified Band Theory Description for CdSe in Solid State

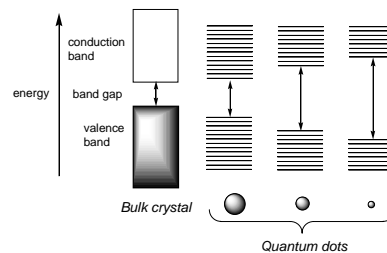
## Band Gap and Color



- When the semiconductor band gap is comparable to the visible light energy
  - photoexcitation of valence band electron to conduction band occurs  $\Rightarrow$  semiconductor becomes colored
- In CdS, band gap  $\sim 2.4\text{ eV}$  corresponding to  $\lambda = 520\text{ nm}$ 
  - light with  $\lambda < 520\text{ nm}$  will be absorbed  $\Rightarrow$  yellow orange color
- For CdSe, band gap  $\sim 1.74\text{ eV}$  corresponding to  $\lambda = 710\text{ nm}$  (red light)
  - all visible light will be absorbed  $\Rightarrow$  dark metallic color

## Quantum Size Effect

- In a micrometer scale, interaction of an infinite number of atomic orbitals within a crystalline solid leads to a continuum of energy levels known as band
- As the size gets smaller to a nanometer scale, fewer orbitals are involved for interaction, the band continuum becomes broken down to quantized energy levels



## CdSe Quantum Dots

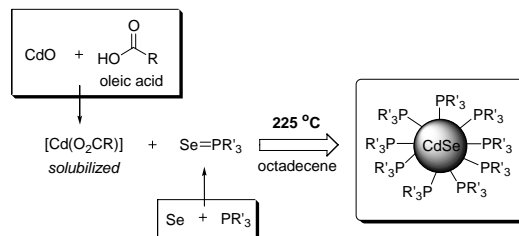
- For micron-sized CdSe crystals, band gap = 1.74 eV  $\Rightarrow$  dark colored solid
- For CdSe QD (1.2 nm), band gap can be widened to  $\sim 2$  eV, corresponding to  $\lambda = 410$  nm (blue-violet light)  $\Rightarrow$  yellow-green color



Fluorescence of CdSe QD under UV exposure

## Making CdSe QDs

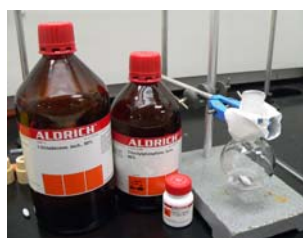
- Starting materials: CdO, elemental Se
- Reagents: Oleic acid, trioctylphosphine (tech grade), octadecene (solvent)



## Key Issues in Making CdSe QDs

- Regular-sized (monodisperse) and -shaped CdSe nanocrystals
- Controlled agglomeration in liquid (colloidal phase) *without* precipitation of large CdSe solids
- Employing trioctylphosphine / trioctylphosphine oxide ligands for
  - stabilizing CdSe nanocrystals in hydrocarbon solvent
  - acting as barrier for spontaneous agglomeration
- Reaction time critical in controlling size of QDs

## Experimental



- Preparation of the Se stock solution

## Experimental



- CdO solution containing oleic acid in octadecene, solution ready for heating to 230 °C

## Experimental



- At 230 °C adding the Se stock solution to the Cd solution, then drawing sample to separate test tubes

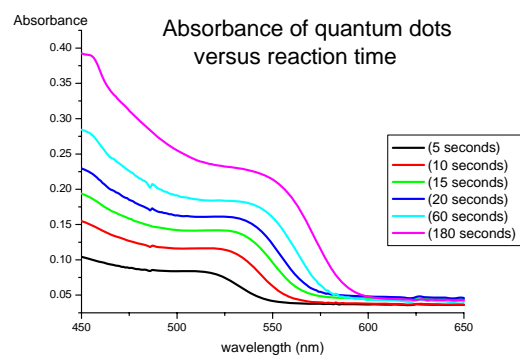


## Experimental



- Variable colors / fluorescence of the CdSe solutions drawn at different time interval

## UV Absorption Spectra



# Preparation and Optical Properties of CdSe Quantum Dot Nanocrystals

## Objective

A major objective in this experiment is to prepare monodisperse CdSe particles. For monodisperse CdSe, the particles exhibit a relatively narrow size distribution and hence display rather distinct absorption and emission peaks.

## Background

CdSe quantum dots demonstrate quantum effect in nanoscale materials. CdSe nanocrystal solutions are colloidal suspensions and that the nanocrystals do not precipitate out of the solution. Individual CdSe quantum dots are composed of a crystalline lattice of Cd and Se atoms. Oleic acid, trioctylphosphine and its oxide may serve as ligands that bind to the exterior of the nanocrystals, allowing the crystals to remain in the octadecene solution. The CdSe quantum dots produced by this method should have their sizes vary from 1.8 – 4.0 nm.

## Materials

1-Octadecene (90% tech), Oleic acid, trioctylphosphine (90% tech), Selenium powder (100 mesh, 99.5+%), cadmium oxide (1 micron, 99.5%)

## Equipment

- 10 mL round bottom flask and stirrer / heater
- Magnetic stirrer bar
- 1 mL syringe (for measuring trioctylphosphine)
- 1 mL syringe (for dispensing selenium stock solution)
- 25 mL round bottom flask and heating mantle
- 10 mL measuring cylinder (for measuring octadecene)
- 1 mL syringe (for measuring oleic acid)
- Mercury thermometer capable of reading 225 °C
- Silicone oil bath
- Ten test tubes and rack
- 9 inch pipets and bulb
- 1 cm glass or polystyrene small volume cuvettes
- Long wavelength ultraviolet light

- Visible spectrometer

## Experimental Procedure

### *Preparation of a Selenium Stock Solution*

To a 10 mL round bottom flask clamped over a stirrer hot plate was added Se (30 mg) and 1-octadecene (5 mL). Using a syringe, trioctylphosphine (0.4 mL) was drawn from its Sure Seal bottle under an atmosphere of nitrogen, and the phosphine was then added to the Se and octadecene mixture. The mixture was stirred with the aid of a magnetic stirrer bar, and the suspension can be gently heated to facilitate dissolution of the Se. The stock solution can be kept at room temperature within a sealed container, and it was ready to use for five preparations.

### *Preparation of CdSe quantum Dots*

To a 25 mL round bottom flask containing oleic acid (0.6 mL) and 1-octadecene (10 mL) was added CdO (0.013 g). The mixture was then heated in a silicone oil bath to 230 °C with stirring over a heater equipped with a magnetic stirrer. Upon complete dissolution of the CdO in the oleic acid / octadecene mixture, a selenium stock solution (1 mL) was added to the clear homogeneous Cd solution at 230 °C. (*N.B. Start timing immediately after addition of the Se stock solution*) Using a 9-inch Pasteur pipette, an approximately 1 mL sample was removed rapidly at frequent intervals and quenched by squirting into separate test tubes. A sampling time at 8, 16, 24, 32, 40, 50, 65, 85, 110 and 140 seconds after the Se addition is recommended. A total of nine or ten samples should be obtained within two to three minutes.

## Hazards

Octadecene vapor would cause irritation by inhalation or contact with the skin and the eyes. Trioctylphosphine is corrosive and causes burns. Oleic acid is air, light and heat sensitive and irritating to the eyes, the respiratory system and the skin. Elemental selenium is toxic by inhalation, by absorption through skin, and the effect of ingestion are cumulative. Cadmium oxide is highly toxic and cadmium compounds are known to be human carcinogens if inhaled or swallowed. All works should be carried out in a fume hood. Protective clothing and gloves should be worn during handling of the reagents.

## References

Boatman, E. M.; Lisensky, G. C.; Nordell, K. "A Safer Easier and Faster Synthesis of CdSe Quantum Dot Nanocrystals" *Journal of Chemical Education*, **2005**, 82, 1697


Yu, W. W.; Peng, X.; "Formation of High Quality Semiconductor Nanocrystals in Non-Coordinating Solvents," *Angew. Chem. Int. Ed.* **2002**, 41, 2368.

 *Experiments on Contemporary Chemistry Topics*


**Chemical Analysis of Gancao (甘草) raw herbs and Commercial products**


**Dr. Daniel K.W. Mok**  
Lecturer  
Department of Applied Biology and Chemical Technology




 **Outline**

- ◆ Therapeutic value of Gancao
- ◆ “Active Ingredients” verses “Markers”
- ◆ Thin Layer Chromatography

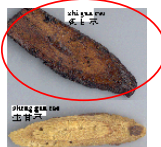

 **Gan Cao**


|                    |                    |   |
|--------------------|--------------------|---|
| <b>Plant Name</b>  | Gan Cao            | <p><b>甘 草</b></p>  |
| <b>Latin Name</b>  | Radix Glycyrrhizae |   |
| <b>Common Name</b> | Licorice           |   |

 **Classification**


**Radix Glycyrrhizae**

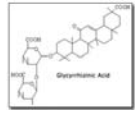
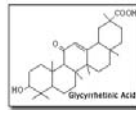
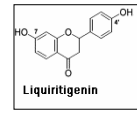
- Different processing methods**
  - Gan Cao (生甘草)
  - Zhi Gan Cao (炙甘草)
- Different sources**
  - Dong Gan Cao (東甘草)
  - Xi Gan Cao (西甘草)
- Different parts of the raw licorice**
  - Pi Gan Cao (皮甘草)
  - Fen Gan Cao (粉甘草)

 **Therapeutic Effects**

- Licorice is sweet in taste, neutral in nature, attributive to the heart, lung, spleen and stomach channels.
- **Indication:**
  - To reinforce the function of the spleen and replenish qi,
  - To remove heat and counteract toxicity,
  - To dispel phlegm and relieve cough,
  - To alleviate spasmodic pain, and
  - To moderate drug actions.

 **Active Ingredients and Markers**

| Glycyrrhizinic acid (GL)   | Glycyrrhetic acid (GA)  | Liquiritigenin (LIQ)  |
|--|---|---|
|    |    |  |
| <ul style="list-style-type: none"> <li>■ Anti-toxic activity</li> <li>■ Immunomodulating effects</li> <li>■ Anti-viral activity</li> </ul> | <ul style="list-style-type: none"> <li>■ anti-tumor</li> <li>■ anti-inflammatory</li> <li>■ anti-allergy (used in cosmetics)</li> </ul> | <ul style="list-style-type: none"> <li>■ Anti-ulcer</li> </ul>                        |



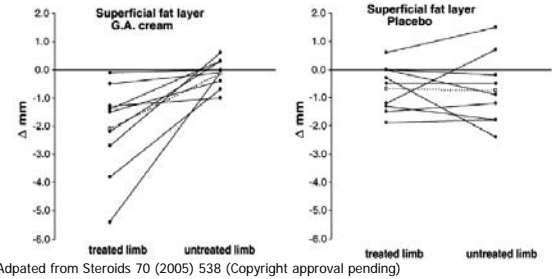
## Glycyrrhetic acid (GA) and its cosmetic uses

Reduce the thickness of superficial subcutaneous fat and the circumference of the thigh of healthy women with normal BMI.



Adapted from Steroids 70 (2005) 538 (Copyright approval pending)

## Glycyrrhetic acid (GA) and its cosmetic uses



## Glycyrrhetic acid (GA) and its cosmetic uses

MAS063D (Atopiclair(R)) is a hydrolipidic cream  
Active ingredients: hyaluronic acid, telmestine, Vitis vinifera, glycyrrhetic acid.

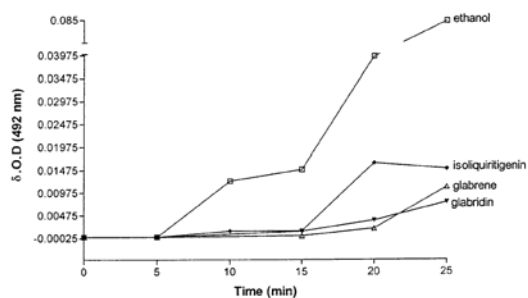
A five-week study in 30 adult patients with mild to moderate AD showed that MAS063D offered significant benefits over a control after 22 days treatment.

Total body area affected (17.2% --> 13.2%,  $p < 0.001$ )  
Itch score (2.7 --> 1.3 on a 10-point scale,  $p = 0.001$ )  
The patients' opinion of MAS063D is significantly better than control ( $p = 0.008$ ,  $p = 0.042$  respectively).

## Ingredients inhibit tyrosinase

| Glaridin | Glabrene | Iso-liquiritigenin |
|----------|----------|--------------------|
|          |          |                    |

## Glycyrrhizae Extracts and Whitening effect



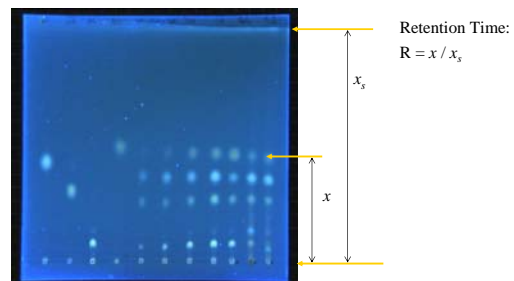
## Raw herbs



## TLC - Introduction



## TLC – analysis



## Standards (Markers)

| Standard Name | R <sub>f</sub> Value |
|---------------|----------------------|
| GA            | 0.90                 |
| LIQ           | 0.57                 |
| GL            | 0.10                 |

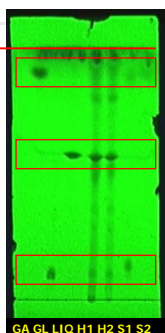
Solvent Front

GA

LIQ

GL

Origin



## Experimental Tips

- Extraction of Raw Herb
- Application of samples on the TLC plate
- Preparation of mobile phase
- Extraction of the Commercial Products
- Application of samples on the TLC plate

## Chemical Analysis of Ganciao (甘草) Raw Herbs and Commercial Products

### Objective

To identify three chemical components of Ganciao raw herbs and commercial products by Thin Layer Chromatography (TLC)

### Introduction

Ganciao is commonly called Licorice Radix in English, and its Latin name is *Radix Glycyrrhizae*. It is a widely used Chinese medicine (CM) for several thousand years and appeared as an “assistant” in many classic formulations. The Latin word *Radix* indicated that the dried root is used. Licorice is divided into two types: the *Pi Ganciao* (皮甘草) and the *Fen Ganciao* (粉甘草). The different of these licorices is that Pi Ganciao is with skin, while Fen Ganciao is without skin.

According to Traditional CM theory and classifications, Ganciao is sweet in taste, neutral in nature, attributive to the heart, lung, spleen, and stomach channels. It can reinforce the function of the spleen and replenish ‘qi’ and remove heat and counteract toxicity. It is also able to relieve spasmodic pain and cough. From modern scientific studies, there are three ingredients found which is able to treat different diseases. Glycyrrhizinic acid (also known as Glycyrrhizin 甘草酸, Fig. 1(a)) has an anti-hepatotoxic activity to treat chronic hepatitis and cirrhosis, and its anti-viral activity has a variety of uses in bronchial problems such as catarrh and coughs. Glycyrrhetic acid (甘草次酸, Fig. 1(b)) has anti-tumor property. Liquiritigenin (甘草素, Fig. 1 (c)) is an anti-ulcer agent and a whitening agent. It is the whitening effect which makes Ganciao extracts being included in cosmetic products such as the White Cream from DHC. These three active ingredients are the target components in this study.

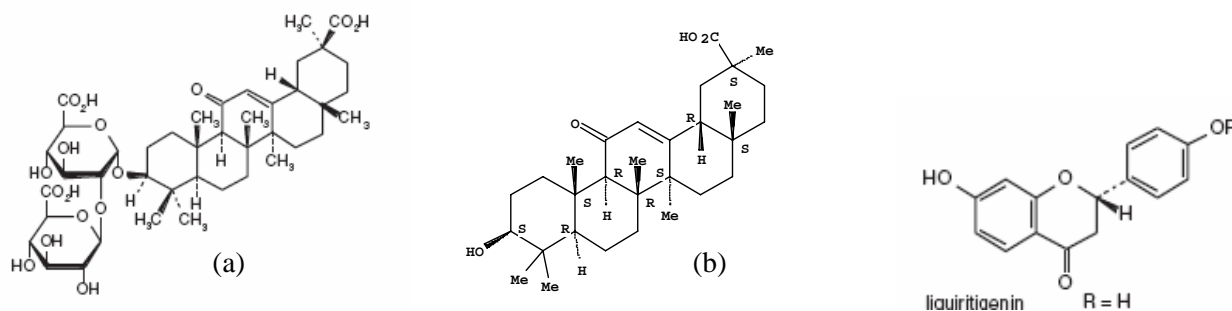


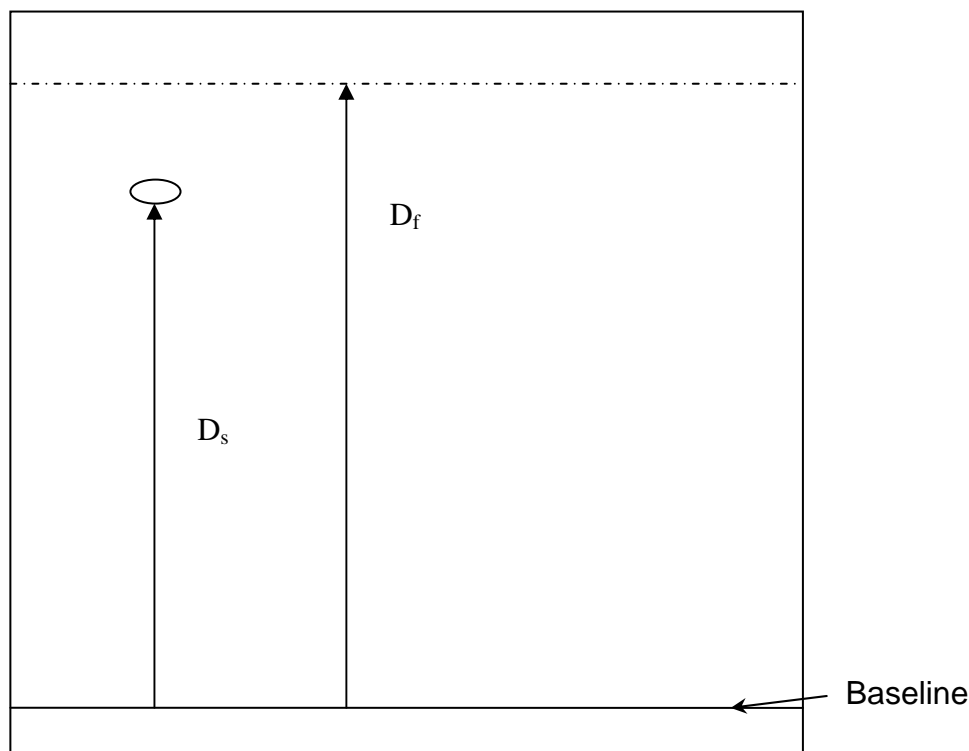
Fig. 1. The chemical structures of (a) Glycyrrhizinic acid, (b) Glycyrrhetic acid and (c) Liquiritigenin

The most common methods of Gancuo chemical analysis are Thin Layer Chromatography (TLC) and High Performancer Liquid Chromatography (HPLC). In this experiment, TLC is employed to analyze the chemical components of Gancuo herbs and commercial products. TLC has been used in the chemical analysis of Traditional Chinese Medicine (TCM) as it is simple and relatively fast. The separation is based on partitioning between mobile (development liquid) phase and stationary phase. The stationary phase is usually silica gel. Nowadays, pre-coated aluminum plates are commonly used. Mobile phase consists of solvents of different polarity. The choice of the mobile phase depends on the sample being analyzed. A sample is spotted onto the plate with a capillary or a micropipette, and the chromatogram is developed by placing the plate in a container with suitable mobile phase. The solvent will elute up the plate, and the sample components will move together with the solvent up the plate at different rate based on their interactions of the stationary phase and mobile phase. After a certain period of time, the plate is taken out from the container to observe the spots that may appear on the plate under visible or UV lamp. The spots are characterized by the distance traveled comparing to the mobile phase, the  $R_f$  value:

$$R_f \text{ values} = D_s / D_f$$

Where  $D_s$  = distance between baseline and a spot

$D_f$  = distance between baseline and solvent front



## Materials

Dichloromethane (AR grade), Ethanol (AR grade), Methanol (AR grade), 25% Ammonia (AR GRADE), Ethyl acetate (AR grade), Glycyrrhizinic acid (99% HPLC), Glycyrrhetinic acid (99% HPLC grade), Liquiritigenin (99% HPLC grade)

## Samples:

- (H1) Fen Gancao (粉甘草) purchased from local drug store such as Yue Hwa
- (S1) DHC Lotion
- (S2) DHC White Cream

## Hazards

90% ethanol, ethyl acetate, and hexane are flammable. 90% ethanol is slightly hazardous in case of skin contact, of eye contact. Ethyl acetate and hexane are hazardous in case of ingestion, of inhalation, of skin contact, of eye contact. Ethyl acetate is toxic to mucous membranes, upper respiratory tract as well.

## Apparatus:

- Capillary tubes
- Forceps
- 600 ml Beaker
- Silica gel pre-coated TLC plate
- Aluminum foil
- Plastic wrap
- [Pencil and Ruler]

## Procedure:

### I. Preparation of the developing solvent

1. Prepare 27ml developing solvent by the following table:

| Solvent         | Volumn (ml) |
|-----------------|-------------|
| Dichloromethane | 12          |
| Ethanol         | 6           |
| Methanol        | 5           |
| 25% Ammonia     | 4           |

2. Pour the developing solvent prepared in step (i) into a 600 ml beaker
3. Seal the opening of the beaker with plastic wrap and aluminum foil to saturate for 30 minutes.



## II. Sample preparation:

➤ Herbal sample (H1) extraction Procedure:

1. Add 1 spatula of sample to a 100ml conical flask
2. Add 5ml ethanol to the conical flask and covered with Aluminum foil.
3. Sonicate for 10 minutes.
4. Filter the sample by suction.

➤ DHC lotion (S1) extraction procedure:

1. 0.5ml of the sample is mixed with 0.5ml ethanol in an eppendorf.

➤ DHC cream (S2) extraction procedure:

1. Add about half spatular of the cream into a 100ml beaker.
2. Add 1 ml ethanol and stir until dissolve.
3. Add 2 ml ethyl acetate and stir until precipitate.
4. Filter the solution by suction.

## III. TLC Development

1. Draw a baseline with pencil at 1 cm above of the bottom of the silica gel TLC plate (5 x 10 cm)
2. Draw 4 cross along the baseline
3. Spot one standard mixture 3 times on the baseline using capillary tubes
4. Spot the H1 sample on the baseline until visible yellowish spot appear
5. Spot 5 drop of S1 and S2 samples onto the baseline as well
6. Put the plate into the 600 ml beaker (Don't move the beaker afterward)
7. Observe the separation of the spots until the solvent front reach below 1 cm of the top

## IV. Data Analysis

1. Observe the result in the UV lamp under 254nm
2. Calculate the  $R_f$  (retardation factor) value of Glycyrrhizinic acid, Glycyrrhetinic acid and Liquiritigenin on the the standard mixture track.
3. Identify Glycyrrhizinic acid, Glycyrrhetinic acid and Liquiritigenin from the three sample extracts