NORTH LEBANON

PHARMA&BIOTECH INDUSTRIES

ASPIRIN PRODUCTION

PENICILLIN / AMPICILLIN

CHEMICAL MATERIALS

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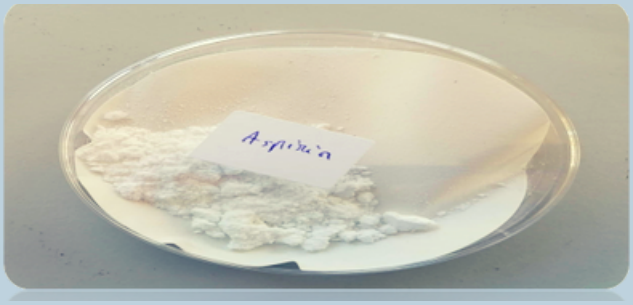
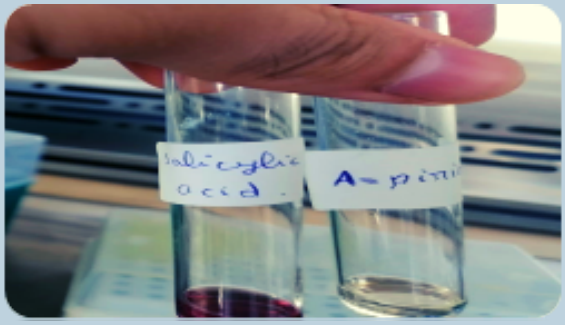
ASPIRIN PRODUCTION AND QUNATIFICATION

LAB SCALE FOR ASPIRIN PRODUCTION
ASPIRIN SYNTHESIS:

•Place 2.027g (0.015 mole) of salicylic acid in a volumetric flask.
•Add 5 mL (0.05 mole) of acetic anhydride, followed by 5 drops of conc. H2SO499% (use a dropper, H2SO4 is highly corrosive)
•Swirl the flask gently until the salicylic acid dissolves.
•Heat the flask gently on the warm bath (65-70°C) for 15 minutes.
•Allow the flask to cool at room temperature.
•Add 10 ml of cold water in 2-3ml portion and cool the mixture for about 10 min in an ice bath so aspirin can start precipitating.
•Add 25 ml of cold water to transform all the crystals to Buchner funnel
•Filtrate the aspirin using filter paper (add small amount of cold water on the filter paper before adding the solution).
•Pour the crystals into a beaker.
•Dissolve them again by adding 40 ml of warm water or 5ml ethanol with warm water.
•Put the beaker in ice to recrystallize.
•Filter the product again.





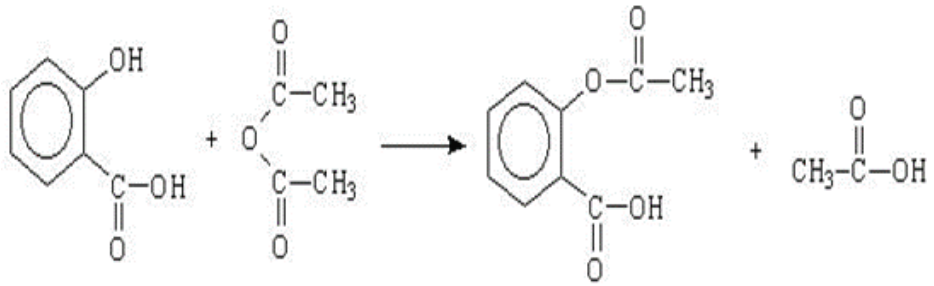
Results of qualitative test of FeCl3 with salicylic acid (purple) and aspirin (transparent)

Note: Iron (III) ion reacts with phenols to form a purple complex. Salicylic acid contains a phenol group, but acetylsalicylic acid does not.

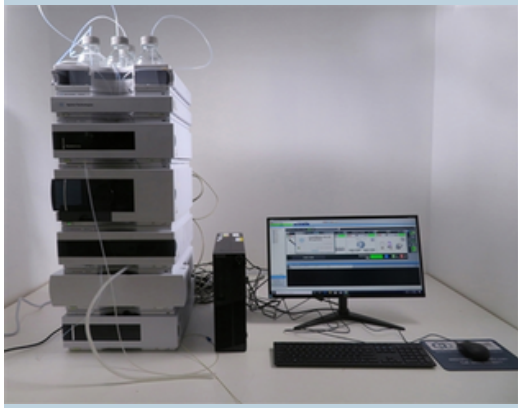
ASPIRIN

Aspirin Quantification :

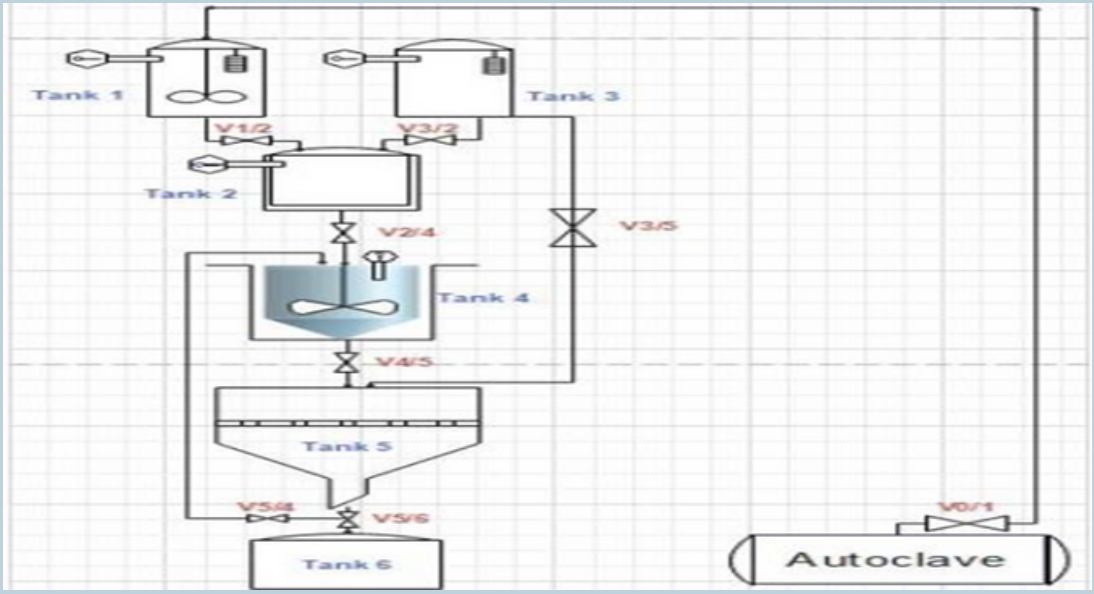
The quantification of the produced aspirin is done with HPLC wish is an analytical chemistry technique used to separate, identify, and quantify each component in the mixture.
First you run pure standard with known concentration and note down retention time and peak area.
Then run sample and note down the chromatographic area of peak appear at same retention time as that of standard.
Calculate concentration= sample Area of sample divided by area of standard multiply by conc. of standard.



Salicylic acid ($C_7H_6O_3$) + Acetic anhydride ($C_4H_6O_3$) → Acetylsalicylic acid ($C_9H_8O_4$) + Acetic Acid ($C_2H_4O_2$)



ASPIRIN PILOT **PLANT**



ASPIRIN PILOT PLANT SYSTEM DESIGN

ASPIRIN PILOT PLANT MECHANICAL REALIZATION

Penicillin Production

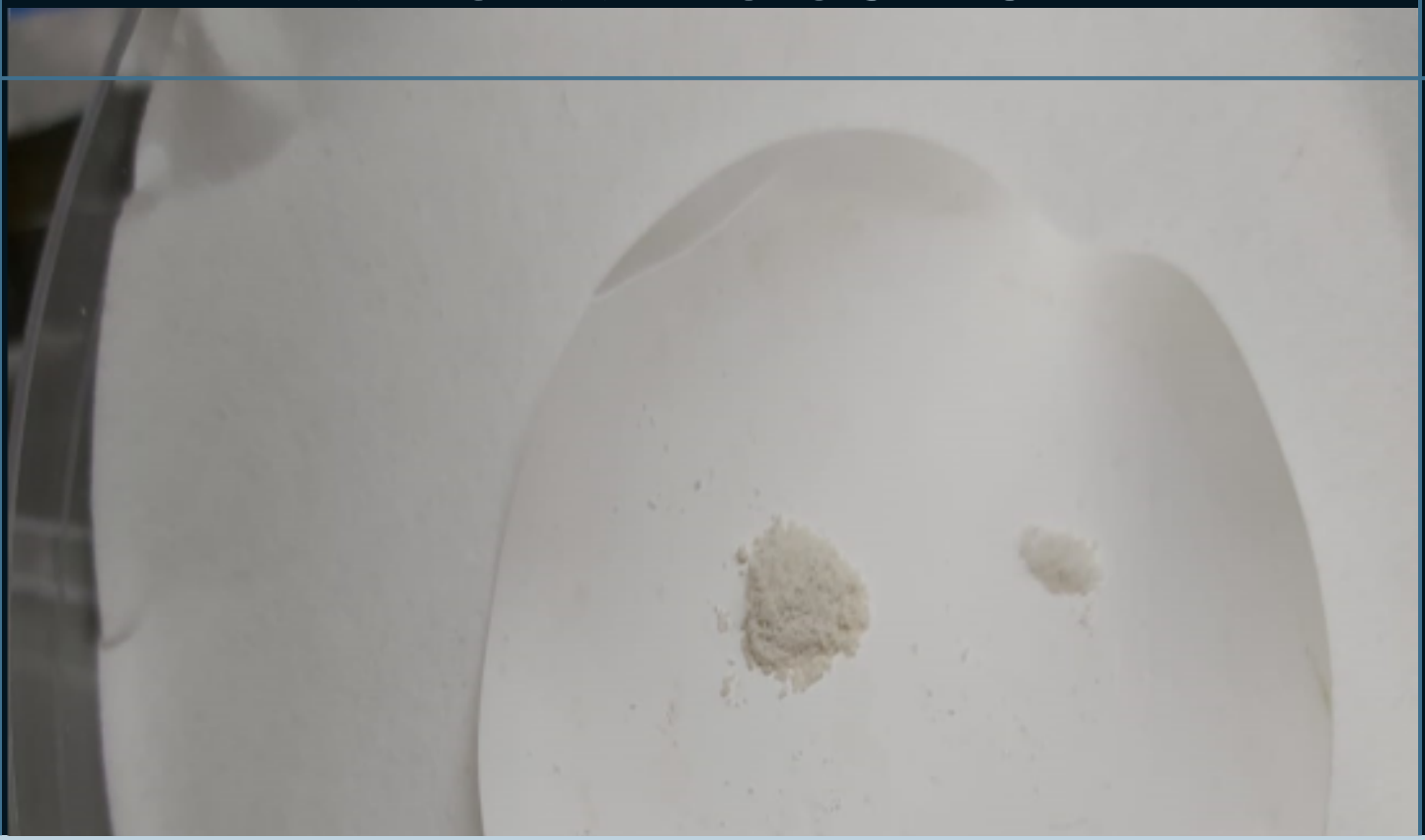
Lab Scale and whole Study of Pilot plant scale Production of Penicillin G With Penicillin Quantification

Introduction

Penicillins (P, PCN or PEN) are a group of antibiotics originally derived from Penicillium molds (principally, P. chrysogenum, P. notatum and P. rubens). The discovery and manufacture of penicillins have changed the face of medicine. The several kinds of penicillin synthesized by various species of the mold Penicillium may be divided into two classes: the naturally occurring penicillins (those formed during the process of mold fermentation) and the semisynthetic penicillins (those in which the structure of a chemical substance-6-aminopenicillanic acid-found in all penicillins is altered in various ways).

Penicillium chrysogenum

PENICILLIN G SODIUM



Lab scale for Penicillin Production:

Preparation of agarose gel:

- We try to melt one tryptone tube by using a heated water (bain marie)
- We weigh 0.5 g of glucose powder
- In an erlenmeyer flask we mix the melted tryptone, the glucose and 10 ml of distilled water
- We heat them in a pressure cooker after boiling for 1 hour
- We let them cool down and we wait about 30 min until the gel are totally solidified
- We put them in the fridge until the time of microbial cultivation

Microbial culture:

- We cultivate the petri dish with the used strain of penicillium
- We incubated them at room temperature for 4 days

Preparation of liquid medium:

- We weigh 2 g of glucose powder, 0.8 g of lactose, 0.8g of peptone, 1g yeast extract, 0.15g of MgCl, 0.15g of KCl, and 0.1g of KH,PO, 0.2g CaCO,, 0.1g corn oil
- It is important to add penicillin precursor which called phenyl acetate at proportion 0.5%
- We add 100ml of distilled water
- We put the mixture in an erlenmeyer flasks
- We heat them with mixing for 15min by using a magnetic hot plate stirrer
- We autoclave the liquid medium, then let it cool down for about 30 min
- We inoculate it with the cultivated petri dish already prepared (about two or three colony)
- We incubate them at room temperature for 7 to 10 days with shaking

Isolation of pure colony:

We took a colony from flie cultivated dish and we isolate it into another tryptone medium to obtain a pure strain that will be used below.

Purification:

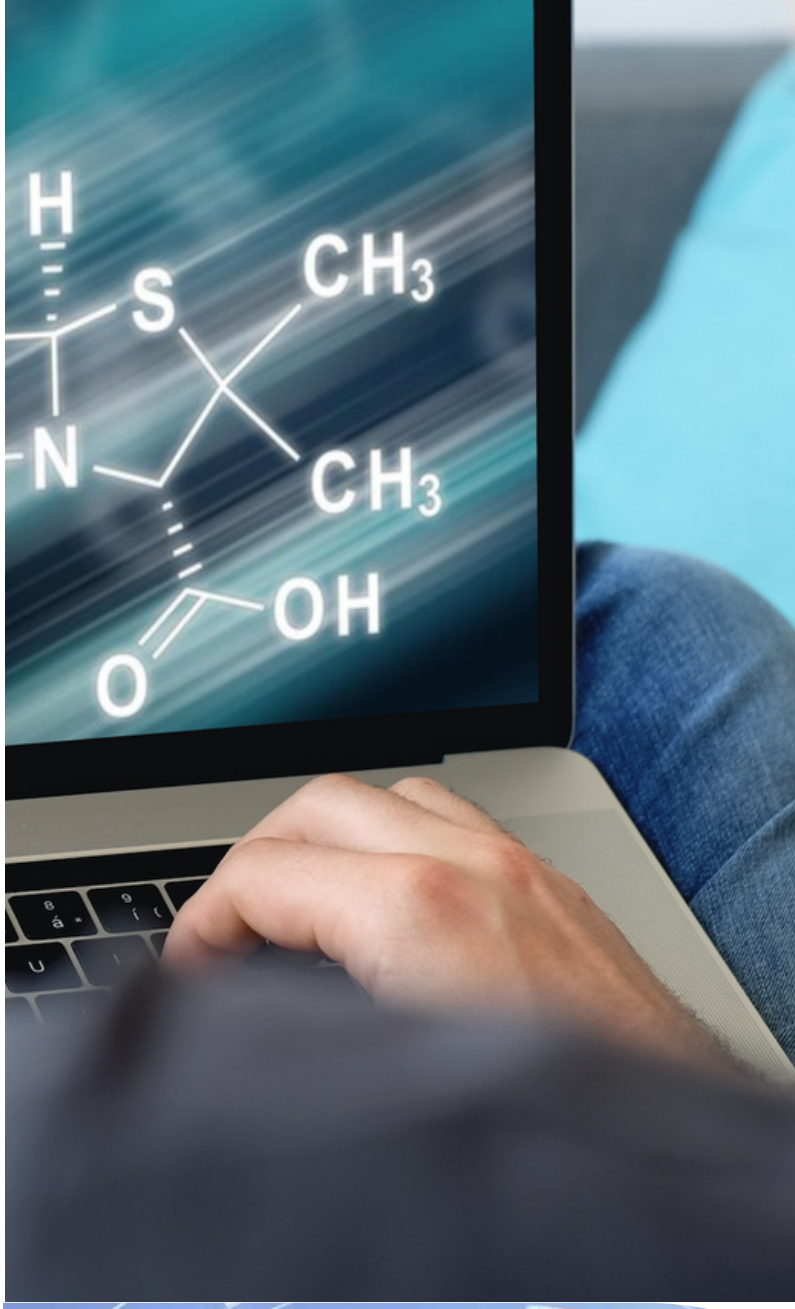
- Harvest liquid medium which contain penicillin by filtration
- Chill to 5-10°C (because penicillin is highly reactive and destroyed by alkali and enzyme)
- Acidify filtrate to PH 2.0-2.5 with H,SO, (to convert penicillin to its anionic form)
- Extract penicillin from aqueous filtrate by adding ethyl acetate (at this very low PH as soon as possible)
- Discard aqueous fraction
- Allow the organic fraction to pass through charcoal to remove impurities and extract penicillin (this step is not important)
- Extract penicillin from ethyl acetate by adding 2% aqueous phosphate buffer (here the PB can be replaced by distilled water) at PH 7.5
- Acidify the aqueous fraction to PH 2-2.5 with mineral acid and re-extract penicillin into fresh ethyl acetate
- Add sodium bicarbonate to the solvent to crystallize the antibiotic as sodium salt
- Recover crystal in filter centrifuge or by filtration.

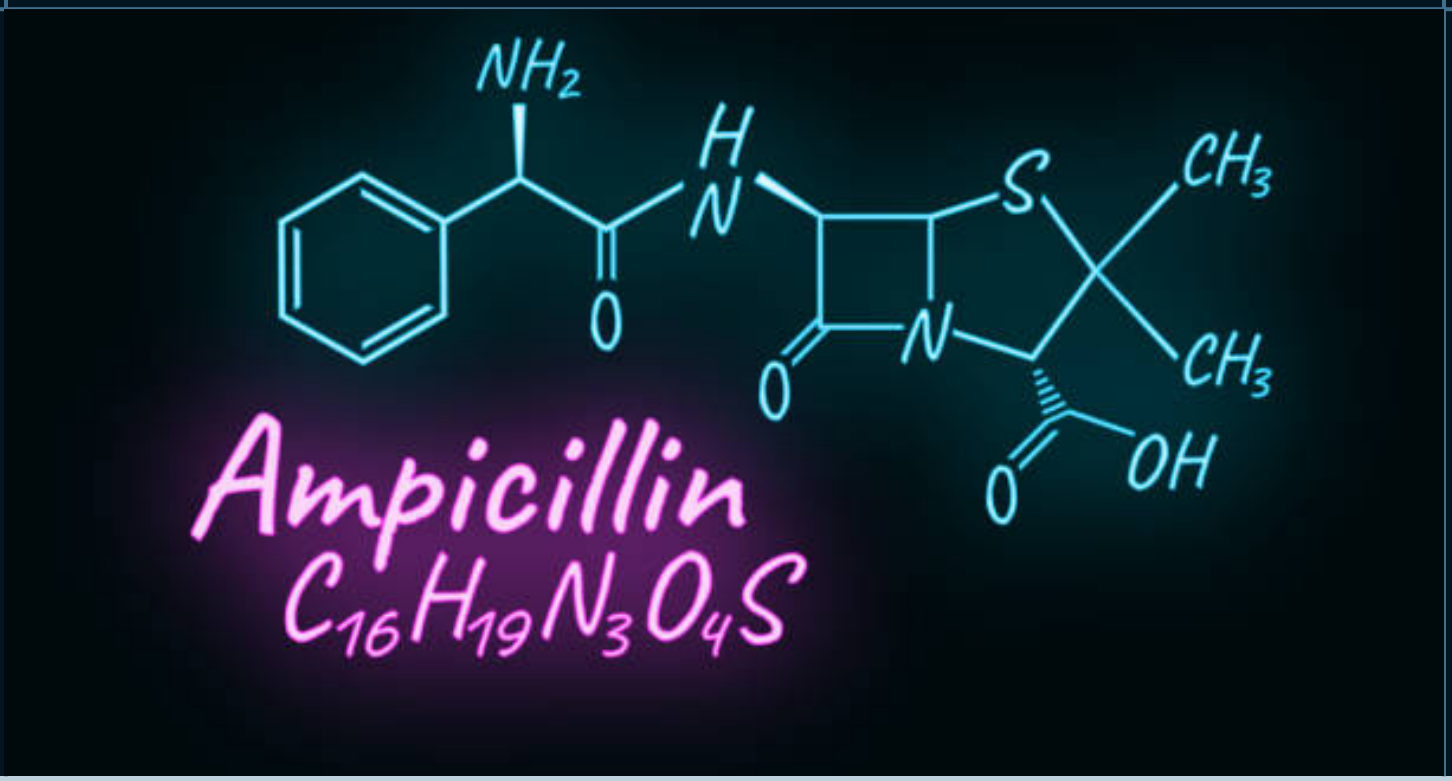
Ampicillin Production

Lab Scale Production of Ampicillin With Ampicillin Quantification

Introduction

Semi-synthetic penicillins antibiotics (SSPAs), one of the most important families of anti-infection drugs in the world market, are mainly producedby a two-step fashion. Ampicillin is one of the most widely used -lactam antibiotics in therapy as it is suitable for a wide spectrum of bacterial infections and has a good level of activity and tolerability. The qualitative analysis was done through B-lactamase test using penicillin or ampicillin resistant Escherichia.coli. Then quantitative analysis was performed by measuring the diameter of zones of inhibition of all the culture samples and comparing them with the standard curve drawn by measuring the diameter of zones of inhibition of standard dilutions of commercially available penicillin G or ampicillin.





Ampicillin production:

To produce semi-synthetic B-lactam(Ampicillin), there are two proposed methods: One put-one step synthesis(1P1S) and one put two steps synthesis(1P2S) while the second has showed a most overall yield then the first.

1- Ampicillin synthesis:

The reaction was carried out in a round bottom flask on a magnetic stir plate at
T° 25° C under constant stirring.

- First, we try to prepare 2 solutions the first is NaOH (1M) and the second is
- H2S04 (1M) which is important to adjust the PH of the reaction
- In order to add penG, We add 7.5 ml of 40 mM of powdered penG by using a phosphate buffer 100mM: PH 7)
- We add 0.8 g of penicillin G acylase (124 UpenG/gram of carrier) or 99.2 UPenG/gram of
- We let the reaction start with stirring and we waited about 60 minutes before the ester was added
- After 60 minutes we add 7.5 ml of 120 mM D-PGMEH(D-Phenylglycine methyl ester hydrochloride)
- Then we add 0.24 g of penicillin G acylase (or 30 Upen G/ gram of carrier)
- We adjust the PH for about 6.4-7.0 by adding NaOH
- We let the reaction continue with stirring
- The second step needs about 1290 min (22.5 h). So the total time needed by the reaction is 1350 min

2- Ampicillin purification and harvesting:

- After the reaction will be finished, H204 is added in order to stop the enzymatic reaction.
- Then a solvent is added (ethyl acetate)
- Sodium bicarbonate solution of 6mM is added to the medium in order to obtain a solid phase.
- The last step is to filter and dry the solution to obtain ampicillin powder as ampicillin sodium
- Her the produced ampicillin is ready to be tested and quantified.

Proposed protocol for ampicillin quantification

1-Preparation of the turbidity calibration 0.5 McFarland(1):

- we add 0.5 mL of a 0.048 mol/L solution of BaCI, (1.175% w/v BaCI, 2H,O) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H,SO, (1% v/v) and we shook vigorously
- We check the density of the suspension using a spectrophotometer with a 1 cm beam and matching cuvettes. The absorbance at 625 nm should be between 0.08 and 0.13
- we distribute the suspension in tubes of the same size as those used to adjust the inoculum and then we seal the tube
- once sealed, we store these tubes at room temperature and protect them from light. Before use, we mix the tube vigorously using a Vortex (6 months' storage)

2- The one bacterial strain which can be used:

E. coli.

3- Quantification of the produced ampicillin using the disk diffusion method:

Preparation of the inoculum:

- We took 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCI 0.9°0)
- We Vortex the saline tube to create a smooth suspension.
- We adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
- Use this suspension within 15 minutes of preparation.

- We inoculate the surface of Mueller Hinton agar plate by streaking the swab 3 times over the entire agar surface, we rotated the plate approximately 60 each time to ensure an even distribution of the inoculum.
- We allow the plate to sit at room temperature at least 3 to 5 minutes (but no more than 15 minutes) for the surface of the agar plate to dry before proceeding to the next step.
- Preparation of the disks: we dilute the standard ampicillin 6 times (Concentrations: 20:15:12;10:8:5 mg/ml) to obtain different concentrations. We distribute 16 disks in plates. On each disk, we add 20 ul of each ampicillin concentration obtained before, in order to have three disk with known concentration and one disk with unknown concentration per plate when we shall add our obtained produced ampicillin.
- Once all disks are in place, we replaced the lid, inverted the plate and placed it at 37°C for 18 to 24 hours.

Chemicals Materials Production

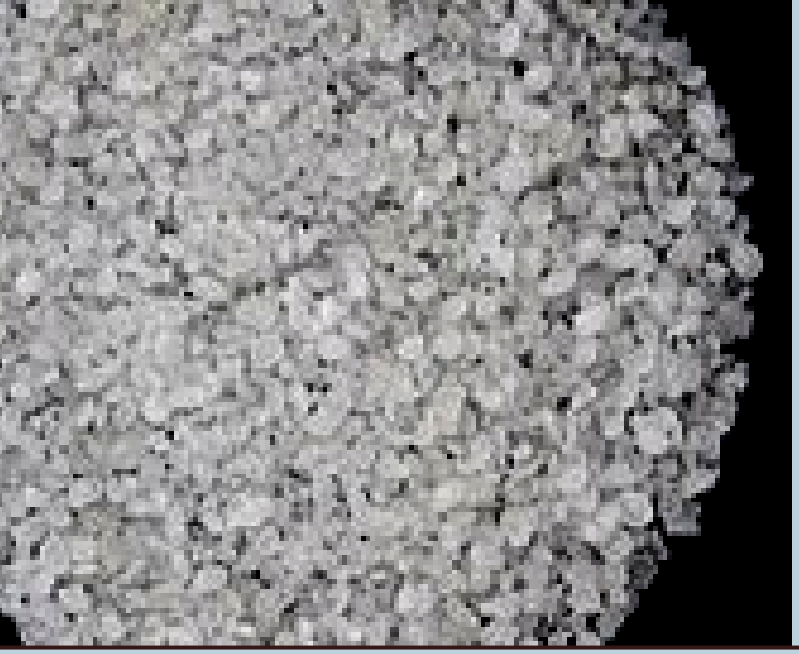
BASIC CHEMICALS MATERIALS FOR ASPIRIN AND PENICILLIN PRODUCTION:

1

SALICYLIC ACID

2

ACETIC ANHYDRIDE



3

PHENYLACETIC ACID

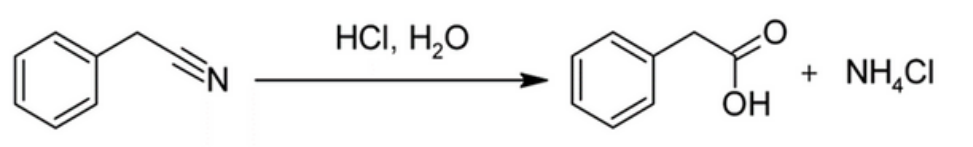
PHENYLACETIC ACID PRODUCTION (PAA-PRECURSOR):

Phenylacetic acid production(PAA-precursor) (aecenar.com)

Phenylacetic acid (PAA) is the precursor needed for a high yield of penicillin G production according to the latest studies, so as per its importance, we are working on its local production.

Different methods of production:

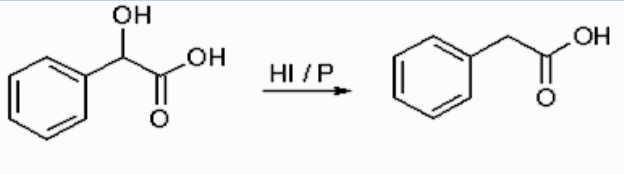
Method 1 – Benzyl cyanide



Reagents and Apparatus:

- Benzyl cyanide
- Hydrochloric acid
- Vacuum distillation apparatusSuction filtration apparatus
- Four necked flask (1000 mL)
- Reflux condenser
- Thermometer
- Dropping funnel

Method 2 - Mandelic Acid (A)



Reagents and Apparatus:

- Mandelic acid
- Potassium iodide (KI)
- Red phosphorous
- Phosphoric acid
- Reflux apparatus
- Ether (for extraction)
- Separatory funnel
- Dilute NaHSO3
- Na2SO4
- Vacuum distillation apparatus

Method 3 - Mandelic Acid (B)

Method 4 - Acetophenone

Method 5 - Styrene

Phenylacetic Acid Synthesis

Introduction

Phenylacetic acid (PAA) is the precursor needed for a high yield of penicillinG production according to the latest sludies. However, as it is required for drug synthesis, its online purchase is restriced. As per its importance, we are working on synthesizing it locally and have compiled this protocol from vanous sources. Its synthesis involves the basic hydrolysis of methyl phenylacetate, followed by its acidification and purification

Materials

- Methyl phenylacetate
- aOH
- HCI
- Diethyl ether
- Anhydrous sodium sulfale

Procedure

- Rellux 2.4g (16 mmol) methyl phenylacetate and 10 ml 2M NaOH for 2h.
- After the solution cools, acidify it with conc. HCI (-3mL). The pH value of the solution must be controlled between 4.0 and 6.0, preferably 5.0
- Extract three times with portions of ether equal to the amount of solution present. The mixture must be stirred until any inorganic precipitate is dissolved.
- Filter the solution and separate the organic layer.
- Wash with cold water.
- Dry over anhydrous sodium sulfate.
- Distill the ether off. Diethyl ether boils at 34.6°C.
- Recrystalize the residue from water to obtain free crystalline phenylacetic acid

Qualification

The quality of the phenylacetic acid is ascertained by determining its melting point using the melting point apparatus.
The melting point of pure phenylacetic acid is from 76 to 77 "C. The closer the value of the product's melting point is to his range and the narrower the range, the purer the product.
Rayane Dergham, Dalia San @ MEGBI/AECENAR Feb 2023
In cooperation

- Dr. samir mourad
- Ceo Mahmoud zoeby
- Supervisor Mohammad kalawoun

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