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TO


Bachelor of Pharmacy
Medicinal Chemistry I
NOTES
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
All Unit in One PDF
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Bachelor of Pharmacy
Pharmaceutical Organic Chemistry III
NOTES
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
All Unit in One PDF
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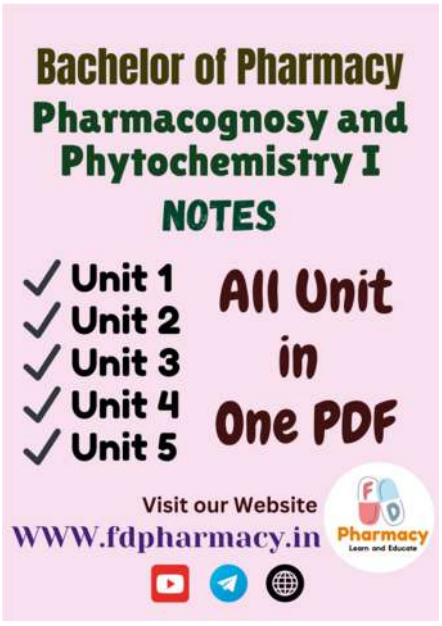
Bachelor of Pharmacy
Pharmacognosy and Phytochemistry I
NOTES
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
All Unit in One PDF
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Bachelor of Pharmacy
Pharmacology I
NOTES
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
All Unit in One PDF
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Bachelor of Pharmacy
Physical Pharmaceutics II
NOTES
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
All Unit in One PDF
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PHARMACOGNOSY AND PHYTOCHEMISTRY – I

UNIT 5

TOPIC :

- **Proteins and Enzymes :** Gelatin, casein, proteolytic enzymes (Papain, bromelain, serratiopeptidase, urokinase, streptokinase, pepsin).

PROTEINS AND ENZYMES

Proteins

Proteins are large, complex molecules made up of amino acids linked by peptide bonds. They are essential for the structure, function, and regulation of tissues and organs in the body.

Examples: Hemoglobin, Insulin, Collagen

Functions:

- Structural component of cells and tissues
- Enzymatic and hormonal regulation
- Transport of oxygen and nutrients
- Immune response (antibodies)

Enzymes

Enzymes are biological catalysts, usually proteins, that accelerate chemical reactions in the body without being consumed. They are highly specific to their substrates.

Examples: Amylase, Lipase, Pepsin

Functions:

- Catalyze biochemical reactions
- Aid in digestion, metabolism, and cellular regulation

Gelatin

Biological Source:

- Gelatin is a protein substance obtained by the partial hydrolysis of collagen.
- Source: Skin, bones, and connective tissues of animals such as cattle, pigs, and fish.
- Collagen is a fibrous protein found in animal tissues.

Preparation / Extraction:

1. Pre-treatment:
    - Animal bones and skins are cleaned and treated with:
        - Acid → Type A gelatin
        - Alkali → Type B gelatin
    - This process breaks down collagen into gelatin.
2. Extraction:
    - The treated material is boiled in water to extract gelatin.
    - The extract is filtered, evaporated, and cooled to form a jelly.
    - Dried into sheets, flakes, or powder.

Evaluation:

1. Physical Tests:
    - Appearance: Colorless to pale yellow; transparent sheets or powder
    - Odor & Taste: Odorless and tasteless
    - Solubility: Soluble in hot water; forms a gel on cooling
2. Chemical Tests:
    - Biuret Test: Violet color confirms protein presence
    - Ash Content: ≤2% (indicates purity)
    - Microbial Limits: Must comply with USP/EP standards

Storage:

- Store in a cool, dry place away from moisture, light, insects, and rodents.
- Use airtight containers to prevent microbial contamination and moisture absorption.

Therapeutic and Pharmaceutical Uses:

- Plasma Expander: Used in emergency fluid replacement (burns, trauma)
- Excipient / Stabilizer: Thickener and base in capsules, lozenges, and suppositories
- Wound Dressings & Skin Care: Provides collagen for tissue repair
- Pharmaceutical Formulations: Acts as gelling, binding, and film-forming agent

Casein

Biological Source:

- **Origin:** Milk of cattle, buffalo, or goats
- **Family:** Not applicable (milk protein)
- **Part Used:** Protein fraction of milk

Preparation / Extraction:

1. Coagulation:
    - Casein is obtained by acidifying milk (using dilute acids like acetic or hydrochloric acid) or by enzymatic coagulation (using rennet or chymosin).
    - This separates the protein from the liquid portion (whey).
2. Purification:
    - The precipitated casein is washed to remove residual lactose and minerals.
    - Dried to obtain casein powder.

Evaluation:

1. Physical Tests:
    - Appearance: White, amorphous powder
    - Odor & Taste: Odorless and bland
    - Solubility: Insoluble in water, soluble in alkali (like NaOH)
2. Chemical Tests:
    - Biuret Test: Violet color confirms protein presence
    - Nitrogen Content: ~15–16%
    - Ash Content: Indicates purity

Storage:

- Store in a cool, dry place in airtight containers
- Protect from moisture, heat, and microbial contamination

Therapeutic and Pharmaceutical Uses:

- Tablet Binder: Acts as a binder in tablet and capsule formulations
- Film Former: Used in coating formulations
- Stabilizer: For emulsions and suspensions
- Nutritional Supplement: Protein source in dietary preparations
- Plasma Substitute Component: Sometimes used in research for protein-rich formulations

Proteolytic Enzymes (Proteases / Peptidases)

Proteolytic enzymes are enzymes that catalyze the hydrolysis of peptide bonds in proteins, breaking them down into smaller peptides or amino acids. They are widely used in medicine, industry, and research.

Key Points

- **Function:** Digest proteins into peptides or amino acids.
- **Site of action:**
  - Stomach (e.g., pepsin)
  - Intestine (e.g., trypsin, chymotrypsin)
  - Lysosomes (intracellular protein degradation)
- **Examples:**
  - **Plant-derived:** Papain (Carica papaya), Bromelain (Ananas comosus)
  - **Microbial-derived:** Serratiopeptidase (Serratia spp.)
  - **Blood/plasma enzymes:** Urokinase, Streptokinase
  - **Animal-derived:** Pepsin (stomach), Trypsin (pancreas)
- **Uses:**
  - Digestive aids for protein malabsorption
  - Wound healing and debridement
  - Anti-inflammatory therapy
  - Fibrinolysis (blood clot breakdown)

Papain

Biological Source:

- **Origin:** Latex from the unripe fruit of *Carica papaya* (Papaya tree)
- **Family:** Caricaceae
- **Part Used: Latex** collected from incisions in the fruit or stem

Preparation / Extraction:

1. **Collection of Latex:**
   - Latex is tapped from incisions in the unripe fruit or stem of papaya.
   - Collected latex is filtered to remove debris.
2. **Purification:**
   - Latex is dried (spray-dried or lyophilized) to obtain papain powder.
   - Sometimes purified by precipitation with alcohol or chromatography.

Evaluation:

1. **Physical Tests:**
   - **Appearance:** Off-white to pale yellow powder
   - **Odor:** Characteristic, slightly sulfurous
   - **Taste:** Bitter, mucilaginous
   - **Solubility:** Soluble in water; forms a viscous solution
2. **Chemical Tests / Activity:**
   - **Proteolytic Activity:** Tested using substrates like gelatin, casein, or albumin
   - **pH:** Active at **pH 6–7**
   - **Enzyme Assay:** Expressed in USP units or casein digestion units
3. **Microbial Limits:**
   - Must comply with USP/EP microbial standards

Storage:

- Store in a cool, dry place, away from moisture and light
- Use airtight containers to maintain enzyme activity
- Protect from heat, which can denature the enzyme

Therapeutic and Pharmaceutical Uses:

- Proteolytic Enzyme: Breaks down proteins into peptides and amino acids
- Digestive Aid: Helps in digestion of dietary proteins
- Wound Care: Used in debridement of necrotic tissue in burns and ulcers
- Anti-inflammatory: Reduces edema in topical formulations
- Pharmaceutical Excipient: Occasionally used in tablet coatings and ointments to enhance protein breakdown

Bromelain

Biological Source:

- **Origin:** Stem and fruit of pineapple (***Ananas comosus***)
- **Family:** Bromeliaceae
- **Part Used:** Stem, fruit, and juice

Preparation / Extraction:

1. **Collection:**
   - Pineapple stems or fruits are crushed to extract juice containing bromelain.
2. **Purification:**
   - Juice is filtered to remove solid debris.
   - Bromelain is precipitated using alcohol, freeze-dried, or spray-dried to obtain powder.
   - Further purification may be done using chromatography for pharmaceutical-grade enzyme.

Evaluation:

1. **Physical Tests:**
   - **Appearance:** Off-white to pale yellow powder
   - **Odor:** Slight characteristic odor
   - **Taste:** Bitter
   - **Solubility:** Soluble in water, forms a viscous solution
2. **Chemical / Enzyme Tests:**
   - **Proteolytic Activity:** Tested using gelatin, casein, or albumin as substrate
   - **pH:** Active around **pH 5–8**
   - **Enzyme Assay:** Expressed in USP units or gelatin digestion units
3. **Microbial Limits:**
   - Should comply with USP/EP standards for microbial contamination

Storage:

- Store in a cool, dry place away from moisture and light
- Use airtight containers to maintain enzyme activity
- Protect from heat, which can denature the enzyme

Therapeutic and Pharmaceutical Uses:

- **Proteolytic Enzyme:** Breaks down proteins into peptides and amino acids
- **Digestive Aid:** Helps in protein digestion in gastrointestinal disorders
- **Anti-inflammatory:** Reduces edema and inflammation in trauma or surgical cases
- **Wound Care:** Aids in debridement of necrotic tissue
- **Pharmaceutical Excipient:** Used in topical formulations and enzyme therapy preparations

Serratiopeptidase (Serratiopeptidase / Serrapeptase / Serratia Peptidase)

Biological Source:

- Origin: Produced by the bacterium Serratia marcescens E-15, originally isolated from the intestine of silkworms (*Bombyx mori*)
- Part Used: Fermentation product of bacterial culture

Preparation / Extraction:

1. **Fermentation:**
    - Serratia marcescens is cultured under controlled conditions to produce the enzyme.
2. **Purification:**
    - The enzyme is separated from the culture medium by filtration.
    - Further purified using precipitation, ultrafiltration, or chromatography to obtain a pharmaceutical-grade powder.
    - Dried to form a stable powdered or granulated form.

Evaluation:

1. **Physical Tests:**
    - **Appearance:** White to off-white powder
    - **Odor & Taste:** Odorless; slightly bitter
    - **Solubility:** Soluble in water; forms a clear solution
2. **Chemical / Enzyme Tests:**
    - **Proteolytic Activity:** Tested using casein, gelatin, or fibrin substrates
    - **pH Range:** Active at **pH 7–9**
    - **Temperature:** Stable at room temperature; denatures at high heat
    - **Units of Activity:** Expressed in USP units
3. **Microbial Limits:**
    - Must comply with USP/EP microbial contamination standards

Storage:

- Store in a cool, dry place away from moisture and light
- Use airtight containers to maintain enzyme activity
- Avoid exposure to heat, which can inactivate the enzyme

Therapeutic and Pharmaceutical Uses:

- Anti-inflammatory: Reduces inflammation and swelling in conditions like arthritis, sinusitis, and post-surgical edema
- Fibrinolytic / Proteolytic: Helps dissolve fibrin and other protein deposits
- Mucolytic: Aids in clearing mucus in respiratory disorders
- Pain Relief: Reduces pain associated with inflammation
- Adjunct Therapy: Used in wound healing, post-operative recovery, and chronic inflammatory disorders

Urokinase

Biological Source:

- **Origin:** Human **kidney cells (renal epithelial cells)** or produced by recombinant DNA technology
- **Part Used:** Secreted enzyme from urine or cell culture

Preparation / Extraction:

1. **Natural Source:**
   - Initially obtained from human urine, which contains the enzyme in small amounts.
2. **Recombinant Production:**
   - Currently, urokinase is produced by genetically engineered **c**ells to yield pharmaceutical-grade enzyme.
3. **Purification:**
   - The enzyme is purified using filtration, precipitation, and chromatography to obtain a stable, sterile product.

Evaluation:

1. **Physical Tests:**
   - **Appearance:** White to off-white powder or lyophilized preparation
   - **Odor & Taste:** Odorless, tasteless
   - **Solubility:** Soluble in water
2. **Chemical / Enzyme Tests:**
   - **Fibrinolytic Activity:** Converts plasminogen to plasmin, breaking down fibrin
   - **pH Range:** Active at pH 7–8
   - **Temperature:** Stable at refrigerated conditions; denatures at high heat
   - **Units of Activity:** Expressed in International Units (IU)
3. **Microbial Limits:**
   - Must comply with USP/EP standards for sterility and microbial contamination

Storage:

- Store in a refrigerated condition (2–8°C)
- Keep in airtight, sterile containers to maintain activity
- Protect from heat and light, which can inactivate the enzyme

Therapeutic and Pharmaceutical Uses:

- **Thrombolytic Agent:** Used in treatment of blood clots such as pulmonary embolism, myocardial infarction, and deep vein thrombosis
- **Fibrinolytic Therapy:** Breaks down fibrin in obstructed blood vessels
- **Adjunct Therapy:** Used in angioplasty and other clot-related interventions
- **Research Tool:** Studied for fibrinolysis and tissue remodeling studies

Streptokinase

Biological Source:

- **Origin:** Produced by β-hemolytic streptococci (e.g., *Streptococcus pyogenes*)
- **Part Used:** Culture filtrate of bacteria

Preparation / Extraction:

1. **Fermentation:**
   - Streptococci are grown under controlled fermentation conditions to produce streptokinase.
2. **Purification:**
   - The enzyme is separated from the culture medium using filtration and precipitation.
   - Further purified by chromatography to obtain a sterile, pharmaceutical-grade enzyme.
   - Can be lyophilized for storage and administration.

Evaluation:

1. **Physical Tests:**
   - **Appearance:** White to pale yellow powder or lyophilized cake
   - **Odor & Taste:** Odorless, tasteless
   - **Solubility:** Soluble in water
2. **Chemical / Enzyme Tests:**
   - **Fibrinolytic Activity:** Converts plasminogen to plasmin, which **breaks down fibrin clots**
   - **pH Range:** Active at **pH 7–8**
   - **Temperature:** Stable under refrigerated conditions; denatures at high heat
   - **Units of Activity:** Expressed in International Units (IU)
3. **Microbial Limits:**
   - Must comply with USP/EP sterility and microbial contamination standards

Storage:

- Store in a refrigerated condition (2–8°C)
- Keep in airtight, sterile containers
- Protect from heat and light, which can inactivate the enzyme

Therapeutic and Pharmaceutical Uses:

- Thrombolytic Agent: Used for treatment of acute myocardial infarction, pulmonary embolism, and deep vein thrombosis
- Fibrinolytic Therapy: Dissolves fibrin in blood clots
- Adjunct Therapy: Used in angioplasty and thrombolytic interventions
- Emergency Medicine: Helps restore blood flow in acute ischemic events

Pepsin

Biological Source:

- **Origin:** Gastric juice of **stomach (chief cells of gastric mucosa)** in mammals such as **pigs, cattle, or humans**
- **Part Used:** Stomach lining (gastric mucosa)

Preparation / Extraction:

1. **Collection:**
   - Gastric mucosa is collected from animals, usually pigs or calves.
2. **Extraction / Purification:**
   - Mucosa is treated with dilute hydrochloric acid (HCl) to activate pepsinogen into pepsin.
   - Extract is filtered to remove solids.
   - Further purified using precipitation, dialysis, or chromatography.
   - Dried to obtain pepsin powder or granules.

Evaluation:

1. **Physical Tests:**
   - **Appearance:** Off-white to yellowish powder or granules
   - **Odor & Taste:** Slight characteristic odor; bitter taste
   - **Solubility:** Soluble in water; forms a viscous solution
2. **Chemical / Enzyme Tests:**
   - **Proteolytic Activity:** Hydrolyzes proteins into peptides
   - **pH Range:** Active in acidic conditions (pH 1.5–3.5)
   - **Temperature:** Stable at moderate temperatures; denatures at high heat
   - **Activity Measurement:** Tested using hemoglobin, casein, or albumin substrates
3. **Microbial Limits:**
   - Must comply with USP/EP microbial contamination standards

Storage:

- Store in a cool, dry place away from moisture and light
- Use airtight containers to maintain enzyme activity
- Protect from heat and moisture, which can denature the enzyme

Therapeutic and Pharmaceutical Uses:

- Digestive Aid: Helps in protein digestion in the stomach
- Enzyme Therapy: Used in peptic formulations and digestive supplements
- Pharmaceutical Excipient: Sometimes used as a proteolytic agent in tablet formulations
- Research Tool: Studied in protein hydrolysis and enzymology experiments