BP403T · SEMESTER IV

BP403T Pharmaceutical Biotechnology

Unit I — Introduction to Pharmaceutical Biotechnology

Final unit notes · Chapters 1–2

From-zero explanations · Pharmacy examples · Exam revision

Pills & Python

Pharmacy • Data • AI • Healthcare

Chapter 1 — Biotechnology in Pharmacy, Protein Therapeutics and Protein Characterization

1.1 Learning Objectives

After studying this chapter, you will be able to:

1. Define biotechnology, pharmaceutical biotechnology and biopharmaceuticals.
2. Explain the difference between small-molecule drugs and protein drugs.
3. Describe the main classes of protein therapeutics with examples.
4. Outline the analytical methods used to characterize proteins.
5. List pharmaceutical applications of biotechnology.

1.2 Definitions

- **Biotechnology:** Using living organisms (or their parts) to make useful products. The word is simple: *bio* (life) + *technology* (tools). A bakery uses yeast (a living organism) to make bread rise — that is ancient biotechnology. A modern version is using bacteria to make the hormone insulin.
- **Pharmaceutical biotechnology:** The branch of biotechnology that uses biological tools to develop medicines.
- **Biopharmaceuticals (biologics):** Medicines made from or in living systems — for example insulin, vaccines, monoclonal antibodies, growth factors.
- **Protein therapeutics:** Medicines that ARE proteins (or protein-like molecules) used to treat disease.
- **Characterization:** Studying a molecule completely so that we know its identity, purity, strength and quality.

1.3 Terminology

- **Polypeptide:** A chain of amino acids. Proteins are long polypeptide chains folded into a shape.
- **Primary structure:** The order (sequence) of amino acids in the chain — like the letters of a word.
- **Secondary/tertiary/quaternary structure:** How the chain folds (secondary), its full 3-D shape (tertiary), and how multiple chains join (quaternary). Shape decides function — like a key must have the right shape to open a lock.
- **Post-translational modification (PTM):** Changes made to a protein after it is built — e.g., adding sugar groups (glycosylation). Very important: the same protein without its sugars may not work properly.
- **Formulation:** The finished medicine product (protein + water + stabilizers) that goes into a vial or syringe.
- **Biosimilar:** A biological medicine that is highly similar to an already approved biologic (not identical, because living systems make slightly different copies each time).

1.4 Explanation (Technical concept → Simple explanation → Example → Key takeaway)

Why biotechnology matters to pharmacy: Many diseases cannot be treated well by small chemical pills. Diabetes needs insulin (a protein), many cancers need antibodies (proteins), and bleeding disorders need clotting factors (proteins). Our bodies only make these naturally in tiny amounts or fail to make them at all. Biotechnology lets us take the human gene for such a protein, put it inside bacteria or animal cells, and get those cells to manufacture the protein in large tanks. The factory is alive — that is biotechnology.

Simple explanation: Think of a small-molecule drug like aspirin as a simple nut you can machine in a factory. Think of a protein drug like insulin as a complex engine that can only be built by a living worker (a cell). Biotechnology trains the worker (cell) to build the engine (protein) exactly right.

Classes of protein therapeutics (with pharmacy examples):

1. **Hormones** — e.g., human insulin (diabetes), human growth hormone (growth disorders).
2. **Enzymes** — e.g., alteplase (dissolves blood clots), L-asparaginase (leukaemia), pancreatic enzymes (digestive aid).
3. **Cytokines and growth factors** — e.g., interferons (viral diseases, some cancers), erythropoietin (stimulates red blood cell production in anaemia), G-CSF (raises white blood cells after chemotherapy).
4. **Clotting factors** — e.g., Factor VIII and Factor IX for haemophilia.
5. **Monoclonal antibodies** — covered fully in Chapter 2 (e.g., trastuzumab for breast cancer, rituximab for lymphoma).
6. **Vaccines and toxoids** — e.g., hepatitis-B vaccine (covered in Chapter 4).

Analytical characterization of proteins — why it is compulsory: A small drug like aspirin is identical every time; you can check it with one simple test. A protein is a giant folded molecule made by living cells. Two batches may differ slightly (different sugar patterns, slight misfolds). So every batch must be characterized with a battery of tests:

1. **Identity tests** — amino acid sequencing and peptide mapping (cut the protein into pieces with an enzyme and check the pattern) to confirm it is the right protein.
2. **Purity tests** — check for contaminants such as host-cell proteins, DNA from the production cell, bacterial endotoxins, and aggregates (clumped proteins).
3. **Structure/shape tests** — circular dichroism and fluorescence to check the folding is correct.
4. **Mass checks** — mass spectrometry to measure exact molecular weight and detect modifications.
5. **Potency/bioassay** — a cell-based or animal test proving the protein actually WORKS, not just looks right.
6. **Stability testing** — checking that the protein stays good during storage (no degradation at the expiry date).

Analogy: Checking a protein batch is like admitting a new student to a hostel: first you check the ID card (identity), then scan the luggage for prohibited items (purity), then check the person is healthy and can do the job (structure and potency).

Key takeaway: Protein therapeutics are complex medicines made by living cells. Because cells are variable, every batch needs a full analytical "health check" — identity, purity, structure, potency and stability — before it can be sold.

1.5 Classifications

- Biopharmaceuticals by source: (a) extracted from natural sources (e.g., insulin from animal pancreas — old method), (b) recombinant (made by engineered cells — modern method).
- Protein drug forms: native proteins, engineered/fusion proteins (two proteins joined, e.g., Fc-fusion proteins with longer half-life), PEGylated proteins (protein + polyethylene glycol chain attached → lasts longer in blood).
- Characterization tests by purpose: identity | purity | structural integrity | potency | stability.

1.6 Mechanisms

How recombinant protein production works (the big picture):

1. Find the human gene for the needed protein.
2. Cut it out and paste it into a circular DNA ring called a plasmid (a vector).
3. Insert the plasmid into a host cell (bacterium *E. coli* or mammalian CHO cell).
4. Grow the cells in large fermenters; the cells read the gene and make the protein.
5. Harvest and purify the protein through many purification steps.
6. Characterize, formulate, fill into vials, and test every batch.

(Details of rDNA technology are in Chapter 3.)

1.7 Diagrams (describe for drawing)

- Draw a comparison: small-molecule drug (tiny hexagon sketch, labelled "simple, identical, one test") vs protein drug (long chain folded into 3-D shape, labelled "complex, slightly variable, many tests").
- Draw the recombinant production pipeline as a 6-step flowchart: Gene → Plasmid → Host cell → Fermenter → Purification → Characterization & fill.

1.8 Flowcharts

Protein characterization workflow: Raw batch → Identity tests (sequencing, peptide map) → Purity tests (host proteins, endotoxin, aggregates) → Structure tests (folding) → Potency (bioassay) → Stability studies → Release or Reject.

1.9 Infographic Concept Box

> 🧬 **BIOTECH IN ONE PAGE:** Living cell = factory. Gene = instruction manual. Protein = product. Biotech medicines (insulin, antibodies, vaccines) treat diseases pills cannot. Every batch gets a 6-point health check: identity, purity, structure, potency, stability, safety. India's biosimilar industry supplies affordable biologics worldwide.

1.10 Tables

Feature	Small-molecule drug (e.g., aspirin)	Protein drug (e.g., insulin)
Size	Very small	Very large (thousands of atoms)
Made by	Chemical synthesis	Living cells
Batch-to-batch variation	Almost none	Small but real (sugars, folding)
Given by	Usually oral	Usually injection (proteins are digested if eaten)
Stability	Very stable	Fragile — needs cold chain

Characterization test	What it answers
Peptide mapping	Is it the right protein?
Mass spectrometry	Exact weight and modifications?
Endotoxin test (LAL)	Any bacterial toxin?
Bioassay	Does it actually work?
Stability study	Will it last till expiry?

1.11 Examples

- A diabetic patient in Chennai uses recombinant human insulin made in *E. coli* — the same hormone her pancreas fails to make.
- Erythropoietin injections help a kidney-failure patient in Mumbai make red blood cells again.

1.12 Analogies

> - Proteins are like hand-knitted sweaters; chemical drugs are like machine-pressed plastic combs. Sweaters need careful checking of every piece; combs come out identical.

1.13 Pharmacy Relevance

- Pharmacists dispense, store and counsel on biologics: cold-chain storage (2–8 °C), no shaking of vials (shaking can unfold proteins), injection technique teaching, and monitoring for allergic reactions.

1.14 India Context

- India is a world leader in affordable biosimilars and vaccines (large manufacturers in Hyderabad, Bengaluru and Pune supply insulin, vaccines and biosimilar antibodies to many countries). The "Pharmacy of the World" title rests partly on biotech manufacturing.

1.15 Key Points

1. Biotechnology = using living systems to make useful products; pharmaceutical biotechnology makes medicines.
2. Protein therapeutics: hormones, enzymes, cytokines, clotting factors, antibodies, vaccines.
3. Proteins are complex and slightly variable → every batch needs full analytical characterization.
4. Characterization covers identity, purity, structure, potency and stability.
5. Proteins are usually injected and need cold-chain storage.

1.16 Common Confusions

- **Biopharmaceutical vs biosimilar:** A biopharmaceutical is any biologic medicine; a biosimilar is a copy of an already-approved biologic (not a "generic" — generics are identical copies, biosimilars are only highly similar).
- **Characterization vs identification:** Identification says "what is it"; characterization is the complete study — identity + purity + structure + potency + stability.

1.17 Common Mistakes

- Writing that proteins can be taken as ordinary tablets — most are destroyed by stomach acid and enzymes, so they are injected.
- Confusing plasmid (the DNA ring used as a tool) with the gene (the instruction it carries).

1.18 Mnemonics

- Protein therapeutic classes: **"HE C-C-MAV"** — Hormones, Enzymes, Cytokines, Clotting factors, Monoclonal Antibodies, Vaccines.
- Characterization 5-point check: **"I-P-S-P-S"** — Identity, Purity, Structure, Potency, Stability ("I Prefer Safe Protein Shots").

1.19 Exam Summary

Biotechnology uses living organisms to make products; in pharmacy it produces biopharmaceuticals such as insulin, growth factors, clotting factors and antibodies. Protein drugs are large, complex and slightly variable between batches, so they undergo rigorous analytical characterization (identity, purity, structure, potency, stability) before release. They are usually injectable and need cold-chain storage.

1.20 One-Minute Revision

⚡ Biotech = living factories making medicines. Protein drugs: hormones, enzymes, cytokines, clotting factors, antibodies, vaccines. Big + complex + variable → 5-point batch check: identity, purity, structure, potency, stability. Inject, don't eat; keep cold.

1.21 Exam Questions

Short answer:

1. Define pharmaceutical biotechnology and biopharmaceuticals.
2. Why can't protein drugs be given orally?
3. List four classes of protein therapeutics with one example each.
4. What is a biosimilar?

Long answer:

1. Explain why analytical characterization of proteins is more demanding than that of small-molecule drugs, describing the main test categories.
2. Describe the classes of protein therapeutics with pharmacy examples and their clinical uses.

MCQs:

1. Which of the following is a protein therapeutic? (a) Aspirin (b) Paracetamol (c) Insulin (d) Ibuprofen — Answer: (c)
2. Peptide mapping is used to confirm: (a) potency (b) identity (c) endotoxin (d) stability — Answer: (b)
3. Biosimilars are: (a) identical chemical copies (b) highly similar biological copies (c) herbal products (d) vaccines only — Answer: (b)

Viva:

1. Why must insulin vials not be shaken? (Shaking can denature/unfold the protein.)

Application-based:

1. A primary health centre reports power cuts. What counselling would you give about storing insulin vials? (Cold chain 2–8 °C, use ice-lined boxes, do not freeze, check expiry and clarity.)

Chapter 2 — Monoclonal Antibodies, Antigens, Enzyme Immobilization and Cell Culture Techniques

2.1 Learning Objectives

1. Define antigens, antibodies and monoclonal antibodies.
2. Describe hybridoma technology for monoclonal antibody production.
3. Explain enzyme immobilization, its methods and advantages.
4. Describe cell culture and cell immobilization techniques.
5. List pharmaceutical applications of antibodies and immobilized enzymes/cells.

2.2 Definitions

- **Antigen:** Any foreign substance that triggers an immune response (a germ, a toxin, or even a part of a protein). Think: *anti-body generator*.
- **Antibody (immunoglobulin):** A Y-shaped protein made by B-cells of the immune system that locks onto a specific antigen.
- **Monoclonal antibody (mAb):** Antibodies made by ONE clone of cells, so every molecule is identical and binds the SAME target spot. ("Mono" = one, "clonal" = from one cell family.)
- **Polyclonal antibodies:** A mixture of antibodies from many cell clones binding different spots — like a crowd throwing many different nets.
- **Epitope:** The exact small spot on the antigen where the antibody binds (the "handle").
- **Immobilization:** Fixing an enzyme or cell onto/into a solid support so it can be reused and does not mix with the product.
- **Cell culture:** Growing animal or plant cells in the laboratory in nutrient liquid (medium) under controlled conditions.

2.3 Terminology

- **Hybridoma:** A hybrid cell made by fusing an antibody-producing B-cell with a cancerous myeloma cell — it makes antibody continuously AND divides forever (B-cell gives the antibody recipe; myeloma gives immortality).
- **Ascites vs in-vitro:** Old method grew hybridomas in mouse belly fluid (ascites); modern method grows them in bioreactor tanks.
- **Carrier/matrix:** The solid material an enzyme is attached to (beads, fibres, membranes).
- **Batch vs continuous:** Batch = make, stop, clean, repeat; immobilized systems allow continuous flow — fresh liquid in, product out, enzyme stays.
- **Primary vs secondary cell culture:** Primary = cells taken directly from tissue (grow a few times); secondary/continuous cell lines = immortal cells that divide indefinitely (e.g., CHO, Vero, HeLa).

2.4 Explanation

Antibodies — the body's guided missiles: When a germ enters, B-cells make antibodies shaped exactly to grip that germ's surface. Each antibody's tip is unique — one lock, one key. In the lab we exploit this precision for both treatment and testing.

Monoclonal antibodies and hybridoma technology (the 4-step story):

1. **Immunize:** Inject the target antigen into a mouse. The mouse's B-cells learn to make the wanted antibody.
2. **Fuse:** Take the mouse's spleen B-cells and fuse them with myeloma (cancer) cells using a fusing agent. Result: hybridomas — cells that both make the antibody and live forever.
3. **Select:** Grow in special HAT medium — only hybridomas survive (unfused B-cells die naturally, unfused myeloma cells are killed by the medium).
4. **Screen and clone:** Test which hybridoma makes the best antibody, then grow that single clone in big tanks. Every antibody molecule is identical — monoclonal.

Simple explanation: A normal immune response is a crowd shouting different instructions (polyclonal). Hybridoma technology picks the ONE best soldier, makes it immortal, and photocopies its perfect weapon millions of times.

Therapeutic monoclonal antibodies (pharmacy examples): trastuzumab (breast cancer), rituximab (lymphoma), adalimumab (rheumatoid arthritis), and many newer ones. They are named with the stem "-mab".

Enzyme immobilization — why fix the enzyme down? Free enzymes mix into the product, get lost, and cannot be reused — expensive. Immobilized enzymes stay put while the reaction liquid flows past: the product comes out clean, and the same enzyme works for weeks.

Methods of enzyme immobilization (4 classic methods):

1. **Adsorption** — enzyme sticks to a surface by weak forces (simplest, but enzyme may leak off).
2. **Covalent binding** — enzyme chemically tied to the support (strong, little leakage).
3. **Entrapment** — enzyme trapped inside a gel or fibre cage (like a bird in a cage — substrate flies in, product flies out, bird stays).
4. **Cross-linking** — enzyme molecules tied to each other into a mesh (no carrier needed).

Cell culture and immobilization techniques:

- **Cell culture basics:** Animal cells need warm (37 °C), sterile, nutrient-rich medium with the right pH and gases. Used to grow viruses for vaccines, to make antibodies, and to test drugs.
- **Cell immobilization:** Whole cells trapped in beads (e.g., calcium alginate beads) or on carriers, so living cells can be reused in a bioreactor for continuous production — used for making antibiotics, organic acids and biotransformation reactions.

Analogy: Free enzyme is like a cook who jumps into the soup — you can't get him out and the soup is contaminated. Immobilized enzyme is like a cook behind a serving hatch — food passes through, cook stays, and he can serve thousands of meals.

Key takeaway: Monoclonal antibodies = identical, single-target antibodies made via hybridoma technology. Immobilization (of enzymes or whole cells) keeps the biocatalyst in place for reuse, cleaner products and continuous manufacturing.

2.5 Classifications

- Antibodies: monoclonal (one clone, one epitope) vs polyclonal (many clones, many epitopes); murine (mouse), chimeric, humanized, fully human (by how "human" the protein sequence is).
- Enzyme immobilization: adsorption | covalent binding | entrapment | cross-linking.
- Cell cultures: primary | secondary (finite) | continuous cell lines (immortal).
- Cell immobilization: entrapment in gels | adsorption on carriers | encapsulation in membranes.

2.6 Mechanisms

Hybridoma selection in HAT medium: HAT blocks the main DNA-building pathway. Cells can survive only via a salvage pathway needing the enzyme HGPRT. Myeloma cells used are HGPRT-negative (they die); normal B-cells have HGPRT but die naturally in days; only fused hybridomas have HGPRT (from the B-cell) AND immortality (from myeloma) → they alone survive.

2.7 Diagrams (describe for drawing)

- Draw the Y-shaped antibody gripping an antigen at the epitope; label Fab tips (binding) and Fc stem.
- Draw the 4-step hybridoma flowchart: Mouse immunization → Cell fusion → HAT selection → Screening & mass culture.
- Draw enzyme immobilization methods: 4 panels — dots stuck on a surface (adsorption), dots tied with strings (covalent), dots inside a net (entrapment), dots tied to each other (cross-linking).

2.8 Flowcharts

Monoclonal antibody production: Antigen → Immunize mouse → Harvest spleen B-cells → Fuse with myeloma → HAT selection → Screen clones → Best clone → Bioreactor scale-up → Purify mAb → Fill & QC.

2.9 Infographic Concept Box

🎯 **GUIDED MISSILES & FIXED COOKS:** Monoclonal antibodies = one clone, one target, endless identical copies (hybridoma: immunize → fuse → select → clone). Immobilized enzymes/cells = catalyst stays, product flows — reusable, clean, continuous. Applications: cancer therapy (trastuzumab), diagnostics (pregnancy kits), industrial biocatalysis.

2.10 Tables

Feature	Polyclonal	Monoclonal
Source	Many B-cell clones	One B-cell clone
Specificity	Multiple epitopes	Single epitope
Batch consistency	Variable	Highly consistent
Production	Simple animal serum	Hybridoma technology

Immobilization method	Strength of attachment	Leakage risk
Adsorption	Weak	High
Covalent binding	Strong	Low
Entrapment	Physical cage	Low (small substrates only)
Cross-linking	Strong	Very low

2.11 Examples

- A pregnancy test kit uses monoclonal antibodies against hCG — one line appears when the antibody catches the hormone.
- Immobilized lactase enzyme is used to make lactose-free milk continuously.

2.12 Analogies

- Epitope and antibody: a specific hook and the exact ring that fits it.
- HAT selection: a sieve that only lets the fused hybrid cells through.

2.13 Pharmacy Relevance

- Pharmacists handle mAb infusions (cold chain, infusion reactions monitoring), dispense diagnostic kits based on antibodies, and advise industries on immobilized-enzyme processes for drug manufacture.

2.14 India Context

- Indian companies manufacture biosimilar monoclonal antibodies (e.g., trastuzumab and rituximab biosimilars) making cancer treatment affordable; government immunization and diagnostic programmes depend on antibody-based kits.

2.15 Key Points

1. Antigen triggers immunity; antibody is the Y-shaped protein that binds it at the epitope.
2. Monoclonal = one clone, one epitope, identical molecules; made by hybridoma technology (immunize → fuse → HAT select → clone).
3. Enzyme immobilization methods: adsorption, covalent binding, entrapment, cross-linking.
4. Immobilization allows reuse, pure products and continuous processing.
5. Cell culture grows animal cells in vitro; cell immobilization reuses whole cells in bioreactors.

2.16 Common Confusions

- **Antigen vs antibody:** Antigen is the invader/target; antibody is the defender protein. (Anti-body generator = antigen.)
- **Monoclonal vs polyclonal:** Mono = one clone/one target; poly = many clones/many targets.

2.17 Common Mistakes

- Saying hybridoma cells are "cancer cells making antibodies" — they are FUSED cells: B-cell (antibody recipe) + myeloma (immortality).
- Mixing up entrapment (enzyme inside a cage) with encapsulation of drugs — different purposes.

2.18 Mnemonics

- Hybridoma steps: **"I-F-S-C"** — Immunize, Fuse, Select, Clone ("I Find Super Clones").
- Immobilization methods: **"A-C-E-C"** — Adsorption, Covalent, Entrapment, Cross-linking ("A Cook Eats Clean").

2.19 Exam Summary

Antigens trigger immune responses; antibodies bind them at epitopes. Monoclonal antibodies are identical single-target antibodies produced by hybridoma technology (mouse immunization, B-cell–myeloma fusion, HAT selection, cloning) and used in cancer therapy and diagnostics. Enzyme/cell immobilization fixes biocatalysts to supports (adsorption, covalent, entrapment, cross-linking) enabling reuse and continuous manufacturing. Cell culture grows animal cells in controlled media for vaccines, antibodies and testing.

2.20 One-Minute Revision

⚡ Antigen = target, antibody = Y-shaped catcher, epitope = handle. mAb: one clone, one target — hybridoma I-F-S-C. Immobilization A-C-E-C: fix enzyme, reuse it, clean product, continuous flow. Cell culture: cells in a bottle; immobilized cells: cells in beads, reused.

2.21 Exam Questions

Short answer:

1. Define antigen, antibody and epitope.
2. What is a hybridoma?
3. List the four enzyme immobilization methods.
4. Why is immobilization preferred in industry?

Long answer:

1. Describe hybridoma technology for monoclonal antibody production with a flowchart.
2. Explain the methods of enzyme immobilization with advantages of immobilized systems.

MCQs:

1. HAT medium selects: (a) B-cells (b) myeloma cells (c) hybridomas (d) T-cells — Answer: (c)
2. The stem "-mab" in a drug name indicates: (a) antibiotic (b) monoclonal antibody (c) vaccine (d) enzyme — Answer: (b)
3. Enzyme trapped inside a gel cage is: (a) adsorption (b) entrapment (c) cross-linking (d) covalent binding — Answer: (b)

Viva:

1. Why are therapeutic antibodies ending in "-mab" given by injection, not orally? (Proteins digested in the gut.)

Application-based:

1. A diagnostic company wants a pregnancy kit that never gives batch-to-batch variation. Monoclonal or polyclonal antibodies — and why? (Monoclonal: single epitope, identical every batch.)

UNIT I — COMBINED REFERENCES

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