MODULE-3

PHARMACY INDIA

Rapid Revision Notes

GPAT BOOSTER

BOOK THAT BOOST UP YOUR GPAT PREPARATION

OTHER SUBJECT

- Biotechnology
- Microbiology
- Pharmaceutical Analysis
- Hospital & Clinical Pharmacy

Features

* According To Latest Syllabus
* All Topics Covered In Concise Forms
* Designed By Pharmacy India Experts
* Important For All Pharma Competitive Exam
* Subject Wise Previous Year Questions

www.pharmacyindia.org pharmacyindia24@gmail.com 8006781759

GPAT

PHARMACY INDIA

Rapid Revision Notes

GPAT BOOSTER

BOOK THAT BOOST UP YOUR GPAT PREPARATION

Module-3

PHARMACY INDIA

DOWNLOAD OUR APP

Download PHARMACY INDIA App from Google Play Store

FOR FREE ONLINE SUPPORT

www.pharmacyindia.org

Contents

SECTION

Biotechnology

➡ Complete Topic

HUMAN GENE THERAPY TRIALS

S.NO.	DISEASE	GENE THERAPY
1.	AIDS	rev and env
2.	Breast cancer	Multidrug resistance I
3.	Citrullinemia	Arginosuccinate synthetase
4.	Colorectal cancer, Melanoma, Renal cancer	Histocompatibility locus antigen-B_7 T(HLA-B_7)
5.	Cystic fibrosis	Cystic fibrosis transmembrane regulator (CFTA)
6.	Diabetes	Glucose transporter-2 (GLUT-2), Glucokinase
7.	Duchenne muscular dystrophy	Dystrophin
8.	Emphysema	α_1-Antitrypsin
9.	Familial hypercholesterolemia	Low density lipoprotein (LDL) receptor
10.	Fanconi anemia	Fanconi anemia C
11.	Gaucher's disease	Glucocerebrosidase
12.	Glioblastoma (brain tumor), AIDS, Ovarian cancer	Thymidine kinase (herpes simplex virus)
13.	Head and neck cancer	P^{53}
14.	Hemophilia B	Factor IX
15.	Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyl transferase (HGPRT)
16.	Melanoma	Tumor necrosis factor (TNF)
17.	Melanoma, Renal cancer	Interleukin-2 (IL-2)
18.	Peripheral artery disease	Vascular endothelial growth factor (VEGF)
19.	Phenylketonuria	Phenylalanine hydroxylase
20.	Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA)
21.	Short stature	Growth hormone
22.	Sickle-cell anemia	β-Globulin
23.	Thalassemia	α-or β-Globulin

ENZYMES USED IN RECOMBINANT DNA TECHNOLOGY/GENETIC ENGINEERING

S.NO.	ENZYME	USE/REACTION
1.	Alkaline phosphatase	Removes phosphate groups from 5'-ends of double single/stranded DNA (or RNA)
2.	Bal 31 nuclease	For the progressive shortening of DNA
3.	DNA ligase	Joins DNA molecules by forming phosphodiester linkages between DNA segments
4.	DNA polymerase I	Synthesizes DNA complimentary to a DNA template
5.	DNase I	Produces single-stranded nicks in DNA
6.	Exonuclease III	Removes nucleotides from 3'-end of DNA
7.	Polynucleotide kinase	Transfers phosphate from ATP to 5'-OH ends of DNA or RNA
8.	Restriction enzymes	Cut double-stranded DNA with a specific recognition site

4.	**Mitochondrial chromosome linked inheritance**	• The inheritance of a trait encoded in the mitochondrial genome. • Persons with a mitochondrial disease may be male or female but they are always related in the maternal line and no male with the disease can transmit it to his children.	• Leber's hereditary optic neuropathy (LHON) • Chronic progressive external ophthalmoplegia (CPEO)

ANALYTICAL METHODS FOR BIOTECHNOLOGY PROCESS VALIDATION

S.NO.	METHODS	DETECTION
1.	Bioassays	• Potency • Tertiary structure of proteins
2.	Carbohydrate analysis	• Glycoforms, • Carbohydrate sequence
3.	Electrophoresis	• Purity, • Impurities, • Glycoforms
4.	HPLC	• Purity and impurities, • Carbohydrate analysis
5.	Mass spectrometry	• Purity and impurities, • Molecular weight, • Glycosylation
6.	Nucleic acid sequencing	• Genetic stability
7.	Polymerase chain Reaction	• DNA, • Viruses, • Mycoplasm
8.	Peptide mapping	• Impurities
9.	Western blot	• Protein impurities

TYPES OF IMMUNE RESPONSES

PRIMARY IMMUNE RESPONSE	SECONDARY IMMUUNE RESPONSE
Slow & short-lived	Prompt, powerful and prolonged
Log phase – Long	Log phase - short
Low-Titer of antibody	High titer of antibody
IgM is secreted	IgG is produced
No negative phase	Negative phase may be seen
Produced after one dose	Produced after multiple doses
Level of antibody is not effective	Level of antibody is effective

IMPORTANT CONTRIBUTIONS IN MICROBIOLOGY

S.NO.	SCIENTISTS	CONTRIBUTIONS
1.	Charles Nicolle	Typhus fever
2.	Enders, Robbins and Weller	Cultivation of Polio viruses
3.	Georges Kohler	Developed hybridoma technology for monoclonal antibodies
4.	Har Gobind Khorana	Interpretation of genetic code
5.	Harald zur Hausen	HPV as cause of cervical cancer
6.	Kary B. Mulis	Polymerase Chain reaction
7.	Marshall & Warren	H. Pylori, its role in Peptic ulcer
8.	Max Theiler	Developed vaccine against yellow fever.
9.	Murray & Thomas	Organ and cell transplantation
10.	Paul Ehrlich and Elie Metchnikoff	Selective theory of antibody formation
11.	Paul H. Muller	Discover DDT's insecticidal action.
12.	Peyton Roux	Viral oncogenesis
13.	Robert Koch	Discovery of causative agent of tuberculosis
14.	Rosalyn Yallow	Developed radioimmunoassay
15	S. Tonegawa	Elucidated the nature of antibody diversity
16.	Sinoussi Montagniar	Discovered HIV virus
17.	Sir Alexander Fleming	Discovery of penicillin
18.	Sir Ronal Ross	Malarial parasite life cycle in mosquitoes
19.	Waksman	Streptomycin (first antitubercular)
20.	Watson and Crick	Double helix structure of DNA

COMPARISION OF CHARACTERISTIC OF PROKARYOTES AND EUKARYOTES

S.NO.	CHARACTERISTIC	PROKARYOTES	EUKARYOTES
1.	Major Group	Bacteria, Blue green algae rickettsiae, chlamydiae, cyanobacteria	Fungi, protozoa, plants, animals, humans
2.	Nucleus	Diffused	Well defined Present with nuclear Membrane
3.	Chlorophyll	Dissolved in cytoplasm (when present)	Contained in chloroplast (when present)
4.	Nuclear Membrane	Absent	Present
5.	Nucleolus	Absent	Present
6.	Ribonucleoprotein	Absent	Present
7.	Cell Division	Binary	Mitosis, Meiosis
8.	Chromosomes	One, Circular	Many, Linear
9.	Extrachromosomal DNA	Found in plasmid	Found in mitochondria
10.	Sterols in Cell Membrane	Absent except in mycoplasma	Present
11.	Cellular Organelles	Absent except ribosomes	Present in a variety of forms
12.	Ribosomes	70S in size	80S in size
13.	Site of Respiration	Mesosome	Mitochondria
14.	Pinocytosis	Absent	Present

			disinfectant. Ex. Alcohol, iodine, etc.
Mechanical Method	Filtration	A. Membrane Filters	Filter through medium of nominal pore size of 0.22 µm or less

CLASSIFICATION OF VIRUS

S.NO.	DNA VIRUS FAMILY	DNA VIRUSES
	DNA VIRUSE	
1.	POXVIRIDAE	Variola Virus, Vaccinia Virus
2.	PAPOVAVIRIDAE	Human Papilloma Virus
3.	PARVOVIRISDAE	Parvovirus
4.	HERPES VIRIDAE	Herpes Simplex Virus 1 And 2 Varicella Zoster, Cytomegalovirus (CMV) Epstein-Barr virus, (EBV)
5.	HEPADNAVIRIDAE	Hepatitis B Virus
6.	ADENOVIRIDAE	Adenovirus
	RNA VIRUS	
1.	PICORNAVIRIDAE	Polio Virus, Hepatitis A Virus Echovirus, Enterovirus, Rhinovirus
2.	CALICIVIRIDAE	Hepatitis E Virus
3.	TOGAVIRIDAE	Rubella Virus
4.	PARAMYXOVIRIDAE	Parainfluenza Virus, Mumps Virus, Measles Virus, Respiratory Virus
5.	ORTHMYXOVIRIDAE	Influenza Virus A, B And C
6.	BUNYAVIRIDAE	Hantavirus, Sandfly Fever Virus
7.	FLAVIVIRIDAE	Dengue Virus, Hepatitis C Virus
8.	CORONA VIRUS	Corona Virus
9.	RHBDOVIRIDAE	Rabies Virus
10.	FILOVIRIDAE	Marburg Virus and Ebola Virus
11.	REOVIRIDAE	Rotavirus, Reovirus
12.	RETRO VIRUS	HTLV (Human T Lymphotropic Virus) HIV (Human Immunodeficiency Virus)

VACCINE USED IN PREGNANT WOMAN, CHILDREN AND INFANTS

VACCINE	ROUTE	DOSE	WHEN TO GIVE
FOR PREGNANT WOMAN			
Tetanus toxoids (TT)-1	Intramuscular	0.5 ml	Early in pregnancy
Tetanus toxoids (TT)-2	Intramuscular	0.5 ml	4 weeks after first Tetanus toxoids-1
Tetanus toxoids booster	Intramuscular	0.5 ml	If received 2 TT dose closes in pregnancy within the last 3 year
FOR INFANTS			
BCG	Intradermal	0.1 ml (00.5 ml for< 1 month)	At birth or as early possible till 1 year.

Hepatitis-B birth dose	Intramuscular	0.5 ml	Within 24 hours
OPV-O	Oral	2 drops	At birth or as early as possible first 15 days
OPV-1,2 and 3	Oral	2 drops	At 6week, 10 week & 14 weeks
Pentavalent 1,2 and 3	Intramuscular	0.5 ml	At 6-week, 10 week & 14 Week (till) 1 year of age
Rotavirus	Oral	5 drops	At 6 weeks, to 10 week & 14 weeks till 1 year of age
IPV	Intradermal	0.1 ml	Two fractional doses at 6 and 14 weeks of age
Measles/MR 1st dose	Subcutaneous	0.5 ml	9 completed months-12 months till 5 year
Vitamin A (1st dose)	Oral	1 ml	At 9 completed months with measles-rubella

SEROLOGICAL TESTS

S.NO.	TEST	DISEASE
1.	Amidase Test	Tuberculosis
2.	Mantoux Test	
3.	Neutral Red Test	
4.	Niacin Test	
5.	Tuberculin Test	
6.	ELISA	AIDS
7.	Karpas Test	
8.	Polymerase Chain Reaction (PCR)	
9.	Reverse Immunoblot Assay	
10.	Western Blot Test For HIV	
11.	Tube Agglutination Test	Typhoid
12.	Widal Test	
13.	Lepromin Test	Leprosy
14.	Kahn Test	Syphilis
15.	VDRL Test	
16.	Wassermann Test	
17.	Elek's Test	Diphtheria
18.	Schick Test	
19.	Dick Test	Scarlet Fever
20.	Schultz Charlton Test	
21.	Cold Hemagglutination Test	Pneumonia
22.	Coombs Test	Brucellosis
23.	Ducrey Test	Haemophilus
24.	Fries Test	Lymphogranuloma Venereum
25.	Ouchterlony test	Small Pox
26.	Radial Immunodiffusion Test	Influenza Virus
27.	Rose Water Test	Rheumatoid Arthritis
28.	Weil Felix Test	Typhus Fever

d) Field Effects

- It has been observed that two functional groups often influence each other's vibrational frequencies by a through-space interaction that may be either steric and/or electrostatic in nature.

Instrumentation

IR radiation source	Incandescent lamp	• Visible –Near IR
	Nerst glower	• Composed of zirconium, yttrium, thorium, heated at Max radiation at 1.4μ temperature range = 1000 - 18000C • **Disadvantage:** emits IR over wide wavelength.
	Globar cell	• Intered silicon carbide, heated at 1300-17000C, max radiation at 1.9μ
	Mercury arc	• Far IR, made up of quartz.
Mono chromators (Grating + prism → order sorter)	Prism	• NaCl (IR) • Lithium/Ca fluoride (region of stretching)
	Grating	• Aluminium, not attacked by moisture. • Use over considerable wavelength.
Sample cells	NaCl, KBr, LiBr, Thorium bromide: Transparent PTFE (Polytetrafluoroethylene).	
Sampling	**Solid run in solution**	• Solid dissolved in non aq solvent.
	Solid film	• (amorphous solids) deposited in cell by evaporation
	Nujol mull	• crystalline solids
		• Solid sample + nujol (mineral oil) → paste • Paste is separated between IR windows • Nujol has IR absorption at 719, 1376, 1462, 2915 cm-1 • Used with hexachlorobutadiene: 760-1010, 1200-1140, 1510 cm-1 • When nujol is used information about C-H stretching. • Alternatively we can use: perfluorohydrocarbon oil, Perchlorobutadiene, perfluorobutadiene, perfluorokerosene.
	Pressed pellet	• Solid sample + KBr → pressed at (25000) • Pellet (up to 2mm thick (0.3mm), (1 cm diameter (13mm) • Always has a band at 3450 cm-1 from OH group.
Detectors	**Thermo couple**	• IR ↑ temp of junction. Due to temp difference between two points potential difference is created leads to flow of electricity.

13C-NMR-spectroscopy

- The 'carbon-skeleton' has been viewed directly with the help of Carbon-13 NMR spectroscopy on a particle basis.
- 13C-NMR refers to recording another NMR-spectrum but of the C-13 atoms rather than the hydrogen atoms.
- In actual practice, however, -'these spectra are recorded in such a manner that each chemically distinct carbon gives rise to single peak, without any coupling or fine structure'.

Applications of NMR

- Assay of drugs
- Drug screening and design
- Native membrane protein
- Metabolite analysis
- Chemical analysis

Mass Spectroscopy

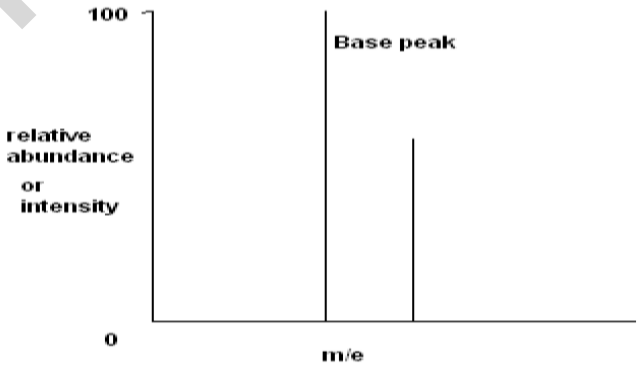
Introduction

- Mass spectrometry is an instrumental technique in which sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their masses.
- Mass spectrum is a plot of relative abundance against the ratio of mass/ charge (m/e).

Basic Principle

- Organic molecules are bombarded with electron → converted into highly energetic positively charged ions (molecular ion or parent ion) → further breakup into smaller ions (fragment ions or daughter ions) → the formed ions are separated by deflection in magnetic field according to their mass/ charge ratio.

$$m/e = H^2r^2/2V$$



- **9-15 eV → parent ions / Mol. Ion peak (M+ = Mol. Wt.)**
- **70 eV**→ fragmentations (fast reaction, excess p. energy) → **Base peak (100% abundance)**

Instrumentation

Sample inlet	
• Solid samples	With low vapour pressure directly inserted into the ionization chamber and volatilization is controlled by heating the probe.
• Liquids	Handled by hypodermic needles injection through a silicon rubber dam.
• Gases sample	Leaked into the ionization chamber directly by the help of mercury manometer.
Ion sources	
• Gas phase ionization	• Electron impact • Chemical ionization • Filed ionization
• Desorption technique	• Field desorption • Electrospray desorption • Matrix assisted laser desorption ionization (MLADI) • Plasma desorption • Fast atom bombardment • Secondary ion mass spectrometry • Thermo spray ionization
Mass analyzers	• Magnetic sector mass analyser
	• Double focussing analyser
	• Quadrupole mass analyser
	• Time of flight analyser
	• Ion trap analyser
	• Ion cyclotron analyser
Detectors	• Faraday cup
	• Electron multiplier
	• Photo-multiplier
	• Micro channel plate

Ion source

MALDI –

- It is a laser ionization mass spectrometry (LIMS) method of vapourizing and ionizing and sample molecules are dispersed in a solid matrix such as nicotinic acid.
- A UV laser pulse removes the matrix which carries some of the large molecules into the gas phase in an ionized form so they can be extracted into a mass spectrometer.

Mass analyzers

- **Quadrupole mass analyser**
  - The quadrupole consist of two pair of parallel rods with applied direct current and RF voltages.
  - Ions are scanned by varying the DC/ RF quadrupole voltages.
- **Time of flight analyser (TOF)**
  - Ions are accelerated through a flight tube and the time of flight to the detector is measured.
  - Typical flight times are 1 to 50micro sec.
- **Vacuum pump:** oil diffusion, mercury diffusion pumps
- **Response time:** 20min-1second
- **Graph:** M/e ratio vs. relative abundance (intensity)
- **Resolution** = mass/mass diff (m/Δm) 1 Part in 20, 1Part in 30000

Thin Layer Chromatography

Introduction

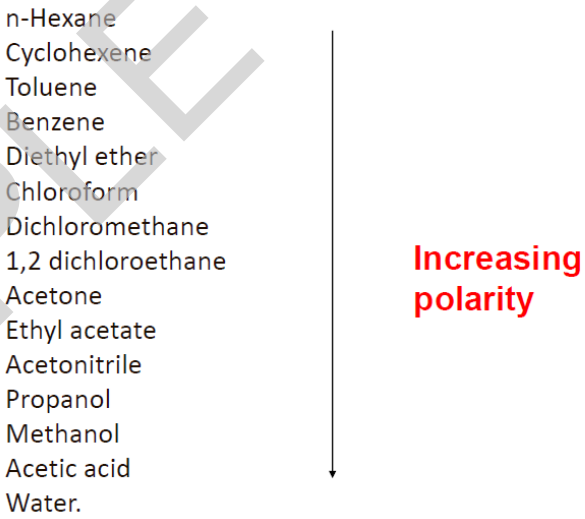
- TLC is one of the simplest, fastest, easiest and least expensive of several chromatographic techniques used in qualitative and quantitative analysis to separate organic compounds and to test the purity of compounds.
- **Thin Layer Chromatography** can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a glass plate and liquid as a mobile phase.
- **Synonyms** → Drop, strip, spread layer, surface chromatography and open column chromatography.
- Separation of the adsorbed substances by the mobile phase.

Principle

- It is based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and nature of solvents employed

Components of TLC

- **Stationary phase** → Silica gel G, Silica gel H, Alumina, Kieselghur
- **Mobile phase** →
  - For example, good separations of polar or ionic solutes can be achieved with a mixture of water and n-butanol.
  - If the stationary phase is hydrophobic, various mixtures of benzene, cyclohexane and chloroform provide satisfactory mobile phases.



Reagents for visualization of TLC chromatograms

Reagents	Application
Iodine vapour	Organic or unsaturated compounds
Phosphomolybdic acid	General organic
Fluorescein/ bromine	General organic
Sulphuric acid	General organic
Ninhydrin	Amino acid
2,4 – dinitrophenylhydrazine	Aldehyde and ketones

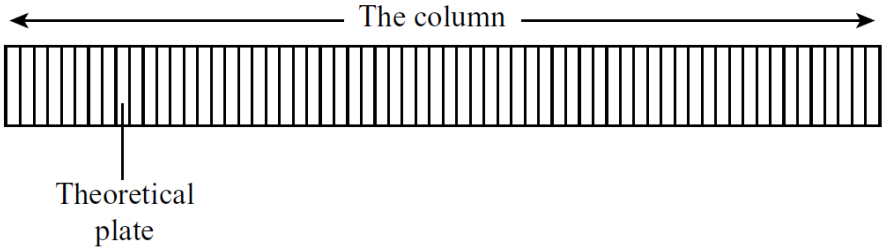
- **Support-coated columns →**the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. It is also known as PLOT (Porous layer open tubular column).

Detectors

Detectors	Type	Support gases	Selectivity	Detectability
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100pg
Thermal conductivity (TCD)	Concentration	Reference	Universal	1ng
Electron capture	Concentration	Make up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50fg
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10pg
Flame photometric	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100pg
Photoionization	Concentration	Make up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2pg
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur	-

Theoretical plate model of chromatography

- The plate model supposes that the chromatographic column is contains a large number of separate layers, called theoretical plates.
- Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates".
- The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.



Oxygen flask combustion method

- Determination of F, Cl, I, sulphur, mercury, phosphorus, arsenic, carbon, and boron etc. in the organic compounds.
- The apparatus is known as Schoniger apparatus.
- Sample holder is ignition gauze.
- Methyl cellulose and gelatin capsu
- le is used for liquid sample.
- Methyl cellulose is more preferred because burn gently, lowers the blank value and no acidic products form.

Kjeldahl Method of Nitrogen Estimation

- Qualitative estimation of organic and inorganic nitrogen in the substances.
- Nitrogen containing organic compound is digested with conc. Sulphuric acid.
- Digestion process (Kinetics) is improved by addition of salts such as Potassium sulphate or sodium sulphate; which increases boiling point of sulphuric acid.
- It's a back titration method. (acid base titration)
- It contains two units: Digestion and steam generation unit.

Thermal Methods

- **Thermal analysis:** the measurement of some physical parameter of a system as a function of temperature.
- **Types of thermal methods-**

Methods	**Parameter measured**	**Graph**
Differential thermal analysis (DTA)	Temperature difference	ΔT v/s temp.
Differential scanning calorimetry (DSC)	Enthalpy	dH/dt v/s temp.
Thermogravimetric analysis (TGA)	Mass	Mass v/s temp.
Dynamic mechanical analysis (DMA)	Deformation	-
Dielectric thermal analysis (DETA)	Deformation	-
Evolved gas analysis (EGA)	Gaseous decomposition	Thermal conductivity v/s temp.
Thermo-optical analysis (TOA)	Optical properties	-

HOSPITAL & CLINICAL PHARMACY

CLASSIFICATION OF HOSPITALS

Type I. On Clinical Basis

CLINICAL-BASIS			NON-CLINICAL-BASIS	
Medicine	**Surgery**	**Maternity**	**Governmental**	**Non-Governmental**
1. Paediatrics 2. Psychiatric and Nervous diseases 3. T.B. 4. General medicine	1. Orthopaedic 2. Gyanaecology 3. ENT	1. Short-term 2. Long-term	-Army hospital -Navy hospital City hospital -Civil hospital -Big hospitals -AIIMS/PGI etc.	Private Hospitals for Profit Non-Profit Church hospital Community hospital Missionary hospital Charitable hospital

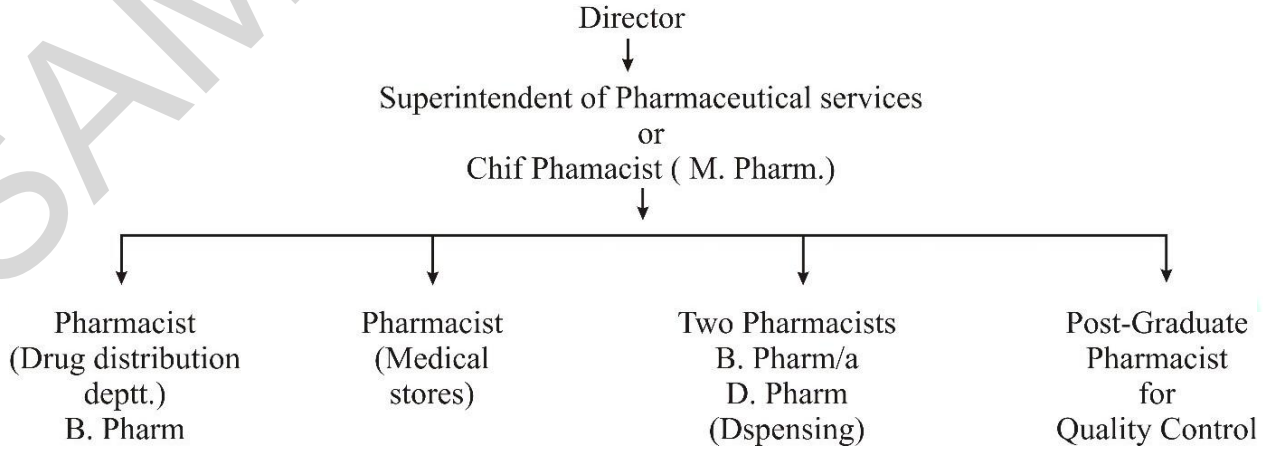
Type II – On size basis

Large hospitals	beds 1000 and above
Medium hospitals	beds between 500 – 1000
Small hospitals	beds between 100 – 500
Very small hospitals	beds less than 100

PHARMACIST REQUIREMENT

BED STRENGTH	NO. OF PHARMACISTS REQUIRED
Upto 50 beds	3
Upto 100 beds	5
Upto 200 beds	8
Upto 300 beds	10
Upto 500 beds	15

REQUIREMENT OF A HOSPITAL PHARMACIST



GPAT-2022 RESULT

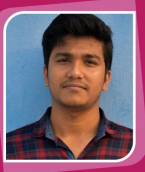
Shining Stars of Pharmacy India

250+ SELECTION

NIKHIL
AIR - 11

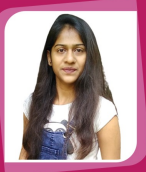
NIKHIL
AIR - 27

ABHISHEK
AIR - 122



SOUMYAJIT
AIR - 126

SUSHANT
AIR - 147



NAMRTA
AIR - 173

SURENDRA
AIR - 192

KRUSHNA
AIR - 204

ADITYA
AIR - 223

YASH
AIR - 223

MAYURI
AIR - 251

AMRENDRA
AIR - 424

AZAR RAZAK
AIR - 468

KHLANDAR
AIR - 497

PRIYANKA
AIR - 556

KAJOL
AIR - 604

SATA DEEP
AIR - 629

ASMA KHANAM
AIR - 651

SUBRAT
AIR - 695

TAVADE
AIR - 795

DIPIN
AIR - 911

ADRIJA
AIR - 958

JOREPALLI
AIR - 1022

NITIN
AIR - 1155

K. MARI
AIR - 1198

PRIYA
AIR - 1198

AMIT
AIR - 1321

RAKESH
AIR - 1361

SEKHAR
AIR - 1404

SUDAM
AIR - 1731

SHIVAM
AIR - 2020

RUDRAWAR
AIR - 2506

NILESH
AIR - 2506

NIRANJAN
AIR - 2613

SAPTAPADI
AIR - 2813

PHARMACY INDIA

Dayalpuram, Street -4, Khatauli
Muzaffarnagar, 251201

Phone : 8171313561, 8006781759

E-mail : pharmacyindia24@gmail.com