NOTESKARTS

COSMETIC SCIENCE

B.Pharmacy 8th Semester

UNIT 3 — Detailed Study Notes

As per AKTU / PCI Syllabus

Sun Protection | Classification of Sunscreens | SPF
Herbs in Skin Care: Aloe Vera & Turmeric | Hair Care: Henna & Amla | Oral Care: Neem & Clove
Analytical Cosmetics: BIS Specifications & Methods for Shampoo, Skin Cream, Toothpaste

SUN PROTECTION AND SUNSCREENS

> Sun protection refers to the use of topical agents (sunscreens) and physical measures to protect the skin from harmful ultraviolet (UV) radiation emitted by the sun, preventing sunburn, photoaging, and skin cancer.

Ultraviolet Radiation — Classification

The sun emits UV radiation in three wavelength bands, each with different skin penetration and damage profiles:

UV Band	Wavelength	Penetration	Biological Effects
UVC	100-280 nm	Absorbed by ozone layer; does not reach Earth	Most dangerous; germicidal; not a concern in normal exposure
UVB	280-315 nm	Penetrates epidermis only	Sunburn (erythema); DNA damage (thymine dimers); primary cause of skin cancer; stimulates melanin production
UVA	315-400 nm	Penetrates deep into dermis	Photoaging (collagen/elastin damage); indirect DNA damage; tanning; contributes to skin cancer; present year-round

UVA Sub-bands	UVA-I (340-400 nm)	UVA-II (315-340 nm)
Penetration	Deepest penetration into dermis	Intermediate — between UVB and UVA-I
Primary Damage	Oxidative stress; photoaging; indirect DNA damage	Erythema; melanin stimulation; closer to UVB effects

UV-Induced Skin Damage — Mechanisms

A. Acute Damage (Sunburn):

- UVB absorption by DNA → thymine dimer formation → p53 activation → apoptosis of damaged keratinocytes ('sunburn cells') → erythema (redness, pain, inflammation)
- **Inflammatory cascade:** UVB → prostaglandins (PGE2), histamine release → vasodilation → erythema within 3-5 hours; peaks at 24 hours

B. Chronic Damage (Photoaging):

- **UVA:** Generates reactive oxygen species (ROS) → oxidative damage to collagen and elastin → wrinkles, loss of elasticity, leathery skin
- **Matrix metalloproteinases (MMPs):** UV activates MMPs → degrade collagen → permanent structural skin damage

- **Photoaging features:** Wrinkles, pigmentation (solar lentigines), telangiectasia, rough texture, loss of elasticity

C. Skin Cancer:

- **Basal Cell Carcinoma (BCC):** Most common; UVB-induced; locally invasive; rarely metastasizes
- **Squamous Cell Carcinoma (SCC):** UVB-induced; p53 mutation; can metastasize
- **Melanoma:** Most dangerous; both UVA and UVB involved; originates from melanocytes

Classification of Sunscreens

> Sunscreens are topical preparations that absorb, reflect, or scatter UV radiation, thereby protecting the skin from UV-induced damage. They are classified by mechanism of action and chemical type.

PRIMARY CLASSIFICATION — By Mechanism of Action:

Type	Mechanism	Examples
Chemical / Organic Sunscreens	Absorb UV radiation and convert it to heat energy through molecular excitation and relaxation (photon absorption)	Avobenzone, Oxybenzone, Octinoxate, Octisalate, Octocrylene, Ecamsule (Mexoryl SX)
Physical / Inorganic Sunscreens	Reflect and scatter UV radiation physically like a mirror; act as a physical barrier on skin surface	Zinc Oxide (ZnO), Titanium Dioxide (TiO2)
Combined (Hybrid) Sunscreens	Use both chemical and physical filters for broad-spectrum protection; most modern commercial sunscreens	SPF 50 products typically combine 2-6 chemical filters + ZnO or TiO2

CHEMICAL SUNSCREENS — Detailed Classification by UV Absorption:

UV Filter	UV Range Covered	Max Conc. (EU)	Key Properties
Avobenzone (Parsol 1789)	UVA-I (360 nm peak)	5%	Gold standard UVA-I filter; photounstable alone — must be combined with Octocrylene or Mexoryl XL for stabilization
Oxybenzone (Benzophenone-3)	UVB + UVA-II	6%	Broad-spectrum; effective; concerns about skin penetration and coral reef toxicity
Octinoxate (Ethylhexyl Methoxycinnamate)	UVB (311 nm peak)	10%	Most widely used UVB filter; photostable; good skin feel; low cost
Octisalate (Ethylhexyl Salicylate)	UVB (307 nm peak)	5%	Mild UVB filter; stabilizes Avobenzone; excellent skin feel; photostable
Octocrylene	UVB + UVA-II	10%	Stabilizes Avobenzone; photostable; film-forming; water-resistant
Homosalate	UVB (306 nm peak)	10%	Good skin absorption; dissolves other UV filters; used in high-SPF products

Ecamsule (Mexoryl SX)	UVA-II (345 nm peak)	10%	Photostable UVA filter; water-soluble; used in L'Oreal products
Bemotrizinol (Tinosorb S)	UVB + UVA-I broad-spectrum	10%	Broadest-spectrum organic filter; highly photostable; not yet FDA approved (EU/Asia approved)
Bisoctrizole (Tinosorb M)	UVB + UVA broad-spectrum	10%	Pigment-like micro-particle; both absorbs and reflects UV; photostable

PHYSICAL SUNSCREENS — Detailed Comparison:

Property	Zinc Oxide (ZnO)	Titanium Dioxide (TiO2)
UV Range	UVB + full UVA-I and UVA-II (broadest spectrum)	UVB + UVA-II; less effective for UVA-I vs ZnO
SPF Contribution	Moderate to high	High UVB contribution; lower UVA
Photostability	Excellent — photostable	Excellent — photostable
Skin Feel	White cast; newer nano/micronized forms reduce whitening	White cast; micronized forms better cosmetically
Skin Sensitivity	Very gentle — approved for sensitive, baby skin	Generally gentle; photo-catalytic activity concern at nano size
Mechanism	Reflects + scatters + some absorption of UV	Reflects + scatters UV
Preferred Use	Baby sunscreens, sensitive skin, reef-safe formulations	Higher SPF products; often combined with ZnO

SPF — Sun Protection Factor

SPF (Sun Protection Factor) is a numerical measure of how effectively a sunscreen protects against UVB radiation (erythema-inducing rays). It indicates the factor by which the MED (Minimum Erythemal Dose) of protected skin is increased relative to unprotected skin.

SPF Formula:

SPF = MED of sunscreen-protected skin / MED of unprotected skin

MED (Minimum Erythemal Dose) = Minimum UV dose that produces just perceptible redness (erythema) 24 hours after exposure

SPF in Practice:

- **SPF 15:** Blocks ~93.3% of UVB; MED is 15x higher with sunscreen
- **SPF 30:** Blocks ~96.7% of UVB; most widely recommended by dermatologists
- **SPF 50:** Blocks ~98% of UVB; preferred for high-UV exposure activities
- **SPF 50+:** Blocks ~98.5-99%; diminishing returns above SPF 50

SPF Classification Table:

SPF Category	SPF Range	UVB Protection	Recommended For
Low Protection	SPF 6-14	75-93%	Darker skin tones; brief sun exposure; very short outdoor activity
Medium Protection	SPF 15-29	93-97%	Daily moisturizer use; moderate outdoor activities; Indian skin tones
High Protection	SPF 30-49	97-98%	Standard recommendation; outdoor sports; beach; fair skin
Very High Protection	SPF 50+	98-99%	Very fair/sensitive skin; extreme UV environments; post-procedure skin

SPF Testing Method (In Vivo):

- **Standard method:** ISO 24444:2019 (International Standard)
- **Test subjects:** 10 human subjects with skin phototype I-III (fair skin)
- **Application:** Sunscreen applied at 2 mg/cm2 (standard dose); compared to unprotected skin
- **UV source:** Solar simulator (xenon arc lamp matching solar spectrum)
- **Endpoint:** MED determined at 16-24 hours post exposure; average SPF calculated from all subjects

UVA Protection — PA System and Critical Wavelength:

UVA Rating System	PA System (Japan/Asia)	Critical Wavelength (USA/EU)
Basis	Based on PPD (Persistent Pigment Darkening) test; UVA-induced tanning	Wavelength at which cumulative absorbance reaches 90% of total absorbance
Rating	PA+, PA++, PA+++, PA++++ (highest)	CW >= 370 nm = 'Broad Spectrum' claim allowed
UVA Protection Factor	UVA-PF >= 1/3 of SPF required for 'Broad Spectrum' in EU	FDA: Broad Spectrum if CW >= 370 nm

Formulation Building Blocks of Sunscreen

Ingredient	Conc.	Examples	Function
UV filter (chemical)	2-10% each	Avobenzone, Octinoxate, Oxybenzone, Octocrylene	Primary sun protection; must have synergistic SPF
UV filter (physical)	2-25%	Zinc Oxide, Titanium Dioxide (micronized)	Broad-spectrum physical protection; photostable
Photostabilizer	1-5%	Octocrylene, Bemotrizinol, Polyester-8	Prevent degradation of photounstable filters (Avobenzone)
Emollient/base	5-30%	Silicones (dimethicone), plant oils, esters	Application vehicle; skin feel; water resistance
Emulsifier	2-8%	Polysorbate 60, Cetyl alcohol, GMS	Create stable O/W or W/O sunscreen emulsion
Film former	0.5-2%	PVP, Acrylates copolymer, DHHB	Improve substantivity; water resistance; even film
Humectant	2-10%	Glycerin, Panthenol, Hyaluronic acid	Skin moisturization; prevent dryness
Preservative	0.5-1%	Phenoxyethanol, Methylparaben	Prevent microbial contamination of aqueous phase
Antioxidant	0.1-0.5%	Vit C, Vit E, Niacinamide	Neutralize ROS generated by UV; complementary photoprotection

Advantages and Disadvantages of Chemical vs Physical Sunscreens

Parameter	Chemical Sunscreens	Physical Sunscreens (ZnO, TiO2)
Skin feel	Lightweight; no white cast; elegant feel	White cast (especially micronized reduces this); may feel heavy
Onset of action	Need 15-20 min before sun exposure to absorb into skin	Immediate protection upon application
Skin sensitivity	Some cause irritation; oxybenzone in FDA safety review	Very gentle; ideal for sensitive, acne-prone, baby skin

Photostability	Many photounstable (Avobenzone); need stabilizers	Highly photostable; no degradation
UVA coverage	UVA coverage variable; Avobenzone provides UVA-I	ZnO provides excellent broad-spectrum UVA coverage
Environmental	Oxybenzone/Octinoxate banned in Hawaii — coral reef toxicity	ZnO: reef-safe; non-nano ZnO preferred ecologically
Regulatory	Multiple filters available; complex regulatory landscape	ZnO and TiO2 only FDA-recognized safe and effective filters

★ EXAM IMPORTANT POINTS

1. Q: What is SPF? Sun Protection Factor = MED protected skin / MED unprotected skin; measures UVB protection
2. Q: SPF 30 blocks what percentage of UVB? 96.7% (SPF 15=93.3%, SPF 50=98%)
3. Q: Difference between UVA and UVB? UVB = sunburn + skin cancer (epidermis); UVA = photoaging + tanning (dermis)
4. Q: What is Avobenzone used for? UVA-I filter (peak 360 nm); photounstable — needs Octocrylene for stabilization
5. Q: Physical sunscreens act by? Reflecting and scattering UV radiation — Zinc Oxide and Titanium Dioxide
6. Q: PA+++ means? UVA protection rating (Japan system); based on Persistent Pigment Darkening (PPD) test
7. Q: What is broad spectrum sunscreen? Protects against both UVA and UVB; critical wavelength >= 370 nm
8. Q: Standard SPF application dose? 2 mg/cm2 of skin surface (testing standard ISO 24444)

ROLE OF HERBS IN COSMETICS

Herbal cosmetics (cosmeceuticals) are formulations combining conventional cosmetic ingredients with plant-derived bioactive compounds that offer therapeutic skin, hair, and oral benefits backed by traditional wisdom and modern scientific evidence.

WHY HERBS IN COSMETICS?

Natural origin — consumer preference for 'clean beauty' and sustainable ingredients

Multi-functional — single herb provides multiple benefits (antioxidant + antimicrobial + anti-inflammatory)

Rich in phytochemicals: polyphenols, flavonoids, alkaloids, terpenes, vitamins

Traditional validation (Ayurveda, Unani, Chinese medicine) + growing scientific evidence

Generally well-tolerated with fewer synthetic preservatives needed

HERBS IN SKIN CARE

ALOE VERA (Aloe barbadensis Miller)

Family: Liliaceae (Asphodelaceae) | Part Used: Leaf gel (inner parenchyma) | Common Names: Ghrita Kumari (Hindi), Korphad (Marathi), Indian Aloe

Botanical Description and Phytochemistry

Aloe vera is a succulent perennial plant with thick, fleshy leaves containing a mucilaginous gel in the inner parenchyma (clear gel) and a yellow latex (aloe latex) just beneath the rind.

Phytochemical Constituents:

Chemical Class	Key Compounds	Skin Benefits
Polysaccharides	Acemannan, Glucomannan, Aloeride	Moisturization; wound healing; immunomodulation; skin barrier repair
Anthraquinones	Aloin, Aloe-emodin, Barbaloin (in latex, NOT gel)	Antimicrobial; anti-inflammatory; NOT used in cosmetics due to photosensitization risk
Vitamins	Vitamin A, C, E, B12, Folic acid	Antioxidant; collagen synthesis (Vit C); anti-aging
Minerals	Calcium, Magnesium, Zinc, Selenium	Enzyme cofactors; skin repair; antioxidant
Enzymes	Bradykinase, Catalase, SOD, Amylase	Bradykinase reduces inflammation; SOD and Catalase neutralize ROS
Amino Acids	20 of 22 essential AAs; proline, hydroxyproline	Collagen precursors; tissue repair
Plant sterols	Campesterol, Beta-sitosterol, Cholesterol	Anti-inflammatory; barrier lipid repair
Salicylic acid	Trace amounts	Mild keratolytic; anti-inflammatory
Lignins	Cellulose-like compounds	Enhanced penetration of other actives into skin

Mechanisms of Action in Skin Care

A. Moisturization:

- **Acemannan and glucomannans:** Large polysaccharides attract and retain water in stratum corneum — humectant action
- **Mucilaginous gel:** Forms a thin occlusive film on skin surface — reduces TEWL

B. Wound Healing:

- **Acemannan:** Stimulates fibroblast proliferation and collagen synthesis; promotes re-epithelialization
- **Glucomannan:** Increases collagen synthesis and cross-linking; speeds healing

- **Bradykinase:** Hydrolyzes bradykinin — reduces pain and inflammation at wound site
- **Magnesium lactate:** Inhibits histidine decarboxylase → prevents histamine formation → reduces itching

C. Anti-inflammatory:

- **Inhibits arachidonic acid pathway:** Reduces synthesis of prostaglandins and thromboxane A2
- **Beta-sitosterol:** Anti-inflammatory plant sterol; inhibits COX pathway
- **C-glycosides (aloesin):** Inhibit tyrosinase; anti-inflammatory; skin-brightening effect

D. Sunburn Relief and Photoprotection:

- **Cooling gel matrix:** Physical cooling on sunburned skin — reduces burning sensation
- **Antioxidants (Vit C, E, SOD):** Neutralize UV-induced reactive oxygen species (ROS) → reduce oxidative damage
- **DNA repair:** Aloeride activates skin cells to produce thymine dimer repair enzymes after UV exposure

Applications of Aloe Vera in Skin Care Formulations

Formulation	Aloe Vera Conc.	Role and Benefit
Moisturizing gel	70-100% pure gel	Base vehicle and primary humectant; lightweight hydration for oily skin
After-sun lotion	10-50%	Soothes sunburn; anti-inflammatory; hydrates damaged skin
Face serum	10-30%	Delivers wound-healing polysaccharides; anti-aging; brightening
Anti-acne cream	5-20%	Antibacterial against P. acnes; anti-inflammatory; reduces post-acne marks
Eye cream	5-15%	Anti-puffiness; soothing; reduces periorbital inflammation
Face mask	50-80%	Intensive hydration; soothing; anti-inflammatory mask for irritated skin
Sunscreen	5-20%	Enhances moisturization; antioxidant protection; after-sun healing

BIS / Regulatory Standards for Aloe Vera in Cosmetics

- **INCI name:** Aloe Barbadensis Leaf Juice / Aloe Barbadensis Leaf Gel / Aloe Barbadensis Leaf Extract

- **Quality markers:** Acemannan content (minimum 10 mg/mL for certified aloe gel); polysaccharide content; color (should be colorless to slightly yellow)
- **Processing:** Gel should be cold-processed; avoid harsh processing that degrades polysaccharides
- **Preservatives needed:** Pure aloe gel is prone to microbial contamination; citric acid + potassium sorbate typically used
- **Standardization:** International Aloe Science Council (IASC) certification; minimum 10% solid content for whole leaf gel

Advantages and Disadvantages of Aloe Vera in Cosmetics

Advantages	Disadvantages
Multi-functional: moisturizing, anti-inflammatory, wound healing, antioxidant in one ingredient	Highly perishable — fresh gel degrades rapidly; requires careful preservation
Suitable for all skin types including sensitive and acne-prone skin	Aloin (anthraquinone in latex layer) can cause photosensitization — must use purified gel only
Clinically proven: burn treatment, psoriasis, sunburn, genital herpes	May cause contact dermatitis in aloe-sensitive individuals (rare but documented)
Excellent skin penetration enhancer for other active ingredients	Quality varies widely between commercial aloe gel suppliers; standardization difficult
Sustainable; widely cultivated globally; low-cost ingredient	Some 'aloe gel' products contain minimal actual aloe — ingredient labeling may be misleading

TURMERIC (Curcuma longa)

Family: Zingiberaceae | Part Used: Rhizome (dried powder, extract, oil) | Common Names: Haldi (Hindi), Haridra (Sanskrit), Indian Saffron

Botanical Description and Phytochemistry

Turmeric is a tropical perennial herb. The rhizome (underground stem) contains curcuminoids, volatile oils, and polysaccharides. It has been used in Ayurveda for 4000+ years for skin brightening, wound healing, and anti-inflammatory purposes.

Phytochemical Constituents:

Chemical Class	Key Compounds	Skin Benefits
Curcuminoids (2-9%)	Curcumin (77%), Demethoxycurcumin (17%), Bisdemethoxycurcumin (6%)	Primary active; anti-inflammatory, antioxidant, anti-aging, wound healing, skin brightening
Volatile oils (3-7%)	Turmerone, ar-turmerone, zingiberene, curlone	Antimicrobial; anti-inflammatory; fragrance; penetration enhancement

Polysaccharides	Ukonan A, B, C	Immunostimulation; wound healing
Flavonoids	Kaempferol, Quercetin	Antioxidant; UV protection; anti-inflammatory
Minerals	Iron, Manganese, Copper, Potassium	Enzyme cofactors; antioxidant support
Sterols	Beta-sitosterol	Anti-inflammatory; skin barrier support

Mechanisms of Action of Curcumin in Skin

A. Anti-inflammatory:

- **NF-kB inhibition:** Curcumin directly inhibits NF-kB (Nuclear Factor kappa-B) — master regulator of inflammatory gene transcription → reduces IL-1, IL-6, TNF-alpha production
- **COX-2 inhibition:** Inhibits cyclooxygenase-2 enzyme → reduces prostaglandin E2 (PGE2) → anti-inflammatory effect comparable to ibuprofen
- **Lipoxygenase inhibition:** Reduces leukotriene synthesis → controls inflammatory cascade

B. Antioxidant:

- **Direct free radical scavenging:** Curcumin donates H+ to neutralize superoxide, hydroxyl, and peroxyl radicals — ORAC value very high
- **Nrf2 activation:** Curcumin activates Nrf2 pathway → upregulates endogenous antioxidant enzymes (SOD, catalase, glutathione peroxidase)

C. Skin Brightening / Anti-pigmentation:

- **Tyrosinase inhibition:** Curcumin inhibits tyrosinase enzyme → blocks melanin synthesis → reduces hyperpigmentation, dark spots, melasma
- **MC1R modulation:** Modulates melanocortin receptor → reduces cAMP-mediated melanin production

D. Anti-aging:

- **Collagen synthesis stimulation:** Curcumin promotes TGF-beta signaling → stimulates fibroblasts to produce Type I and Type III collagen
- **MMP inhibition:** Inhibits matrix metalloproteinases (MMP-1, MMP-3, MMP-9) → prevents collagen degradation → anti-wrinkle effect

E. Wound Healing:

- **Angiogenesis promotion:** Curcumin promotes new blood vessel formation at wound site → faster healing
- **Keratinocyte migration:** Promotes migration and proliferation of keratinocytes — re-epithelialization of wounds

Applications of Turmeric in Skin Care Formulations

Formulation	Turmeric Content	Role and Benefit
Face pack / mask	2-10% turmeric powder	Traditional Indian skin brightening ritual; anti-inflammatory for acne; Haldi ceremony
Brightening serum	0.1-0.5% curcumin extract	Tyrosinase inhibition; anti-pigmentation; antioxidant glow serum
Anti-acne formulation	0.5-2% turmeric extract	Antibacterial against P. acnes; anti-inflammatory; reduces acne scars
Anti-aging cream	0.1-1% curcumin	Collagen stimulation; MMP inhibition; antioxidant protection
Wound healing cream	1-5% turmeric extract	Promotes re-epithelialization; anti-inflammatory; antimicrobial protection
Sunscreen formulation	0.5-1% curcumin	Supplementary UV protection via antioxidant ROS neutralization; complement to SPF
Soap and body wash	0.5-3% turmeric powder/extract	Skin brightening; antimicrobial; anti-inflammatory; traditional use

Challenges of Turmeric/Curcumin in Cosmetic Formulation

- **Yellow staining:** Curcumin is intensely yellow — stains skin, nails, and clothing; limits use in leave-on products; rinse-off products preferred
- **Poor water solubility:** Curcumin is lipophilic (poorly water-soluble); requires solubilizers (polysorbates, cyclodextrin, nanoemulsions)
- **Photostability:** Curcumin degrades in UV light → products must be in opaque packaging
- **Low bioavailability:** Poor skin penetration of curcumin; nanotechnology (nanocurcumin, liposomes) used to improve penetration
- **pH sensitivity:** Curcumin is red-orange at alkaline pH and yellow at acidic pH — color changes with formulation pH

Advantages and Disadvantages of Turmeric in Cosmetics

Advantages	Disadvantages
Potent antioxidant, anti-inflammatory, and skin brightening in one ingredient	Intense yellow pigment stains skin and clothing — limits leave-on application
Extensive traditional use validation (Ayurveda, 4000+ years) + modern scientific evidence	Poor water solubility; requires complex formulation strategies

Anti-pigmentation via tyrosinase inhibition — alternative to hydroquinone	Photodegradation in UV light; unstable in transparent packaging
Antibacterial against P. acnes, S. aureus, E. coli	Risk of contact dermatitis (rare); nasal sensitivity to turmeric powder
Anti-aging: collagen stimulation + MMP inhibition	Low skin penetration of curcumin; nanotechnology needed for cosmeceutical efficacy

HENNA (Lawsonia inermis)

> Family: Lythraceae | Part Used: Leaves (dried powder) | Common Names: Mehndi (Hindi), Maruthani (Tamil), Mignonette Tree

Botanical Description and Phytochemistry

Henna is a flowering plant whose leaves contain the primary dye molecule lawsone. The dried leaf powder mixed with water, lemon juice, or tea creates a paste that binds to keratin protein in hair, nails, and skin.

Chemical Class	Key Compounds	Hair Benefit
Naphthoquinones	Lawsone (2-hydroxy-1,4-naphthoquinone) — 0.5-1.5% in leaves	PRIMARY dye molecule; binds to keratin; orange-red color; anti-fungal; protein protection
Flavonoids	Luteolin, Apigenin, Quercetin, Kaempferol	Antioxidant; UV protection; anti-inflammatory scalp effect
Tannins	Gallotannins, Ellagitannins	Protein cross-linking; hair hardening; coating and strengthening cuticle
Phenolic acids	Gallic acid, Ellagic acid	Antioxidant; antimicrobial; anti-inflammatory
Polysaccharides	Galactomannan	Conditioning; film-forming on hair shaft
Terpenoids	Linalool, beta-ionone	Fragrance; antimicrobial
Alkaloids	Lawsarindin	Mild antimicrobial; scalp health

Chemistry of Henna Hair Coloring — Lawsone Mechanism

How Lawsone Colors Hair:

- **Step 1:** Henna paste (acidic pH 4.5-6 from lemon juice/vinegar) applied to hair shaft
- **Step 2:** Lawsone (2-hydroxy-1,4-naphthoquinone) releases from plant cell material in acidic aqueous environment

- **Step 3:** Lawsone molecules (small, lipophilic) penetrate through open cuticle scales into cortex
- **Step 4:** Lawsone binds covalently to keratin protein — specifically to the SH groups of cysteine amino acid residues in cortex keratin
- **Step 5:** Lawsone-keratin complex absorbs light → orange-red color; complex is permanent until hair grows out or degrades
- **Color variation:** Pure henna = red-orange; combined with Indigo (Indigofera tinctoria) = brown/black; combined with cassia = blonde/golden

Hair Strengthening and Conditioning by Henna

- **Tannins:** Form cross-links with hair proteins → increased hair shaft thickness and tensile strength → reduced breakage
- **Protein binding:** Henna coats and fills gaps in cuticle → smoother hair shaft → reduced friction → less tangling
- **pH normalization:** Henna paste (acidic) closes cuticle scales → smooth, shiny appearance
- **Antifungal:** Lawsone has proven antifungal activity against Malassezia — natural anti-dandruff property
- **Scalp cooling:** Traditional use as scalp coolant (anti-inflammatory tannins reduce scalp inflammation)

Applications of Henna in Hair Care

Application	Form Used	Benefit
Natural hair dye	Powder paste (fresh/pre-mixed)	PPD-free permanent color; red-orange shade; safe for sensitive scalp
Conditioning treatment	Henna hair mask (with oils/eggs)	Strengthens; reduces breakage; adds shine
Anti-dandruff treatment	Henna paste + tea tree oil	Lawsone antifungal; tannins soothe scalp
Compound henna dye	Henna + Indigo powder blend	Brown to black shades; PPD-free alternative to oxidative dye
Henna-infused shampoo	Henna extract 1-5%	Mild color maintenance; conditioning; scalp health
Pre-shampoo treatment	Henna paste 30-60 min	Deep conditioning; protein bonding; color boost

Advantages and Disadvantages of Henna

Advantages	Disadvantages
100% natural and safe — no PPD allergy risk (unlike chemical dyes)	Limited color range: only orange-red naturally; brown/black requires indigo addition

Permanent coloring — lawsone binds covalently to keratin	Long processing time: 1-4 hours application required for good color
Conditions hair while coloring — tannins strengthen hair shaft	Cannot lighten hair — can only add warm red/brown tones; cannot produce blonde/platinum
Antifungal: controls dandruff naturally (lawsone vs Malassezia)	Stains skin, scalp, nails temporarily; difficult to remove splashes
No chemical damage — no ammonia, no hydrogen peroxide	'Black henna' in tattoos contains PPD — potent allergen; NOT natural henna
Strengthens and thickens hair shaft via tannin-protein cross-linking	Difficult to apply conventional chemical color over henna — chemical color may not penetrate henna-coated shaft

AMLA (Phyllanthus emblica / Emblica officinalis)

Family: Phyllanthaceae | Part Used: Fruit (fresh, dried, powder, extract, oil) | Common Names: Amla, Indian Gooseberry, Amalaki (Sanskrit), Awla (Hindi)

Botanical Description and Phytochemistry

Amla is a deciduous tree bearing small, round, greenish-yellow fruits. It is the richest natural source of Vitamin C (ascorbic acid) and a Rasayana herb in Ayurveda known for anti-aging, hair growth promotion, and strengthening properties.

Chemical Class	Key Compounds	Hair Benefit
Vitamin C (Ascorbic acid)	Very high (445-1000 mg/100g); stable form (emblicanin A and B)	Collagen synthesis; antioxidant; strengthens hair; prevents premature graying
Tannins	Emblicanin A, Emblicanin B, Punigluconin, Pedunculagin	Unique tannins stabilize Vit C; protein cross-linking; hair strengthening; antioxidant
Polyphenols	Gallic acid, Ellagic acid, Chebulinic acid	Antioxidant; anti-inflammatory; anti-graying; DHT inhibition
Flavonoids	Quercetin, Kaempferol, Rutin	Antioxidant; UV protection; anti-inflammatory scalp
Alkaloids	Phyllantine, Phyllembein	Anti-graying; antioxidant properties
Amino acids	Glutamic acid, Proline, Aspartic acid	Keratin protein synthesis; hair strength
Fatty acids (seed oil)	Linoleic acid (36%), Oleic acid (23%), Palmitic acid (14%)	Deep conditioning of hair shaft; scalp nourishment

Mechanisms of Action in Hair Care

A. Hair Growth Promotion:

- **5-alpha reductase inhibition:** Amla extract inhibits 5-alpha reductase enzyme → reduces conversion of testosterone to DHT (dihydrotestosterone) → prevents androgenetic alopecia (male pattern baldness)
- **Dermal papilla stimulation:** Amla promotes proliferation of hair follicle dermal papilla cells (DPCs) — key signal for anagen initiation
- **Wnt/beta-catenin pathway:** Activates Wnt signaling in hair follicle → promotes anagen phase entry → longer growth phase

B. Anti-graying:

- **Melanocyte protection:** Antioxidants (Vit C, tannins) protect melanocytes from oxidative damage → preserve melanin production → reduce premature graying
- **Hydrogen peroxide clearance:** Catalase enzyme supplemented by amla antioxidants → prevents H2O2 accumulation in hair bulb → H2O2 bleaches melanin causing graying

C. Scalp Health:

- **Antimicrobial:** Gallic acid and ellagic acid inhibit Malassezia growth → anti-dandruff
- **Anti-inflammatory:** Polyphenols inhibit NF-kB and COX pathways → reduce scalp inflammation and itching
- **Scalp circulation:** Traditional scalp massage with amla oil improves blood microcirculation → better nutrient delivery to follicles

D. Hair Conditioning:

- **Tannin-protein interaction:** Amla tannins bind to keratin → smooth cuticle surface → improved shine and reduced frizz
- **Vitamin C:** Essential for collagen synthesis → stronger hair follicle anchoring in dermis

Applications of Amla in Hair Care Products

Product	Amla Content	Role and Benefit
Amla hair oil	5-20% amla fruit extract + carrier oil	Traditional hair growth; scalp nourishment; anti-graying; conditioning
Anti-hair fall shampoo	1-5% amla extract	5-AR inhibition; scalp anti-inflammatory; antioxidant scalp protection
Hair growth serum	2-5% amla extract	Dermal papilla stimulation; Wnt pathway activation; anagen promotion
Hair conditioner	1-3% amla extract	Tannin-keratin bonding; smooths cuticle; adds shine; reduces frizz

Amla powder hair mask	5-20% pure amla powder	Traditional deep conditioning; anti-dandruff; natural color maintenance
Herbal hair pack	Amla + henna + shikakai blend	Combined hair growth + coloring + conditioning + cleansing in Ayurvedic tradition

Advantages and Disadvantages of Amla in Hair Care

Advantages	Disadvantages
Richest natural source of Vitamin C (stable form); powerful antioxidant	Vitamin C (ascorbic acid) is unstable in aqueous formulations; oxidizes easily
5-alpha reductase inhibition — natural DHT blocker for hair loss prevention	Unique tannin structure stabilizes Vit C in amla but commercial extraction may lose this synergy
Traditional validation: Ayurvedic Rasayana; thousands of years of use	Raw amla has very sour, astringent taste — poor organoleptics in rinse-off products
Anti-dandruff (Malassezia inhibition) + hair growth + conditioning in one ingredient	Amla oil has strong odor — masking fragrance needed in commercial formulations
Dermal papilla cell stimulation — promotes anagen phase in hair cycle	May slightly darken hair color over time — unsuitable for blonde/gray hair maintenance

HERBS IN ORAL CARE

NEEM (Azadirachta indica)

> Family: Meliaceae | Part Used: Leaves, Bark, Twigs (Datun), Seed oil | Common Names: Neem (Hindi), Nimba (Sanskrit), Margosa Tree, Indian Lilac

Botanical Description and Phytochemistry

Neem is a fast-growing tropical evergreen tree native to the Indian subcontinent. The twigs (datun/miswak) have been used as natural toothbrushes for 4000+ years. Neem contains over 150 biologically active compounds.

Chemical Class	Key Compounds	Oral Care Benefit
Limonoids (Tetranortriterpenoids)	Azadirachtin, Nimbin, Nimbinin, Nimbolide, Gedunin	Primary active class; antibacterial, anti-inflammatory, antifungal; azadirachtin is the benchmark limonoid
Terpenoids	Azadirone, Nimolicinol, Epoxyazadiradione	Antibacterial; bitter taste deters bacteria
Flavonoids	Quercetin, Kaempferol, Rutin, Myricetin	Antioxidant; anti-inflammatory; anti-plaque biofilm

Tannins	Catechin, Epicatechin, Gallic acid	Astringent; tighten gum tissue; antibacterial; anti-plaque; anti-hemorrhagic
Fatty acids (seed oil)	Oleic acid (52%), Palmitic acid (16%), Stearic acid (18%)	Antimicrobial; anti-inflammatory; used in neem seed oil
Polyphenols	Nimandial, chlorogenic acid	Antioxidant; anti-inflammatory; protect oral mucosa
Alkaloids	Nimbidine, Mahmoodin	Antibacterial; anti-ulcer properties

Mechanisms of Action in Oral Care

A. Antibacterial — Anti-plaque:

- **Cell membrane disruption:** Nimbolide and nimbinin alter bacterial cell membrane permeability → leakage of cellular contents → bactericidal
- **Inhibits S. mutans:** Neem extract inhibits glucosyltransferase (GTF) enzyme in S. mutans → prevents sucrose-dependent adhesion to tooth surface → anti-caries
- **Biofilm disruption:** Neem extracts disrupt dental plaque biofilm formation and maturation — effective against both early and mature biofilm
- **Spectrum:** Active against S. mutans, S. salivarius, Lactobacillus acidophilus (caries pathogens); P. gingivalis, F. nucleatum (gingivitis pathogens)

B. Anti-gingivitis:

- **Tannin-astringent action:** Tannins precipitate proteins in gingival sulcus → vasoconstriction → tighten gum tissue → reduce bleeding
- **Anti-inflammatory:** Nimbin inhibits COX and LOX pathways → reduces PGE2 and leukotrienes → anti-gingivitis
- **Antifungal:** Active against Candida albicans — relevant for denture stomatitis and oral candidiasis

C. Tooth Strengthening (Datun mechanism):

- **Physical abrasion:** Neem twig fibres provide mechanical plaque removal — acts like a toothbrush at cellular fiber level
- **Fluoride-like effect:** Neem contains trace fluoride (0.2-0.5 mg/100g); mineral compounds may contribute to remineralization
- **Salivary stimulation:** Chewing neem twig stimulates saliva flow → increases salivary pH → protective buffering against acid attacks

Applications of Neem in Oral Care Products

Product	Neem Content	Benefit
Neem toothpaste	0.5-5% neem leaf extract/bark extract	Anti-plaque; anti-gingivitis; antibacterial against S. mutans; natural fresh breath
Herbal mouthwash	0.5-2% neem extract	Anti-plaque rinse; anti-gingivitis; anti-halitosis; complement to brushing
Neem tooth powder	20-50% neem powder	Traditional formulation; abrasive + antibacterial; all-in-one powder
Oil pulling base oil	Pure neem oil (diluted)	Traditional Ayurvedic oil pulling — antimicrobial oil swishing for oral health
Anti-gingivitis gel	2-5% neem extract	Targeted gingival application; anti-inflammatory; reduces bleeding
Neem tooth gel for children	0.5-1% neem extract	Safe, mild antibacterial for children's oral care; no harsh chemicals

Advantages and Disadvantages of Neem in Oral Care

Advantages	Disadvantages
Broad-spectrum antibacterial against S. mutans, P. gingivalis, Lactobacillus — multi-pathogen coverage	Intensely bitter taste — low palatability; patient compliance issues; requires heavy flavor masking
Anti-plaque + anti-gingivitis + anti-caries in one natural ingredient	Neem seed oil has very strong, pungent odor — difficult to mask in cosmetic formulations
Traditionally used as datun (twig chewing) for 4000+ years in India and Africa	Clinical evidence for neem vs commercial fluoride toothpaste still emerging
Safe for children (no toxicity risk unlike fluoride at high doses)	Some individuals report contact sensitivity to neem in oral care products
Antifungal activity against Candida — useful for denture stomatitis	Standardization of active content (nimbolide, azadirachtin) in commercial extracts is challenging

CLOVE (Syzygium aromaticum / Eugenia caryophyllata)

Family: Myrtaceae | Part Used: Flower buds (dried), Essential oil | Common Names: Laung/Lavang (Hindi), Lavangam (Sanskrit), Caryophyllus aromaticus

Botanical Description and Phytochemistry

Clove is the dried flower bud of a tropical evergreen tree native to Indonesia. Clove oil (obtained by steam distillation) contains the highest concentration of eugenol of any plant essential oil (72-90%). It is used in dentistry and Ayurveda as an analgesic, antiseptic, and anti-inflammatory.

Chemical Class	Key Compounds	Oral Care Benefit
Phenylpropanoids	Eugenol (72-90%), Isoeugenol, Methyl eugenol	PRIMARY active; analgesic (dental pain), antiseptic, anti-inflammatory, antibacterial
Sesquiterpenes	Beta-caryophyllene (5-12%), Alpha-humulene	Anti-inflammatory; analgesic; contributes to clove aroma
Acetyleugenol	Eugenol acetate (acetylated form, 5-15%)	Antibacterial; contributes to aroma
Flavonoids	Kaempferol, Quercetin, Myricetin, Rhamnetin	Antioxidant; anti-inflammatory; protect gum tissue
Tannins	Ellagitannins, Gallotannins	Astringent; tighten gum tissue; antibacterial
Oleanolic acid	Oleanolic acid (triterpene)	Anti-inflammatory; antiplaque; hepatoprotective when ingested
Eugeniin	Hydrolyzable tannin	Antiviral (Herpes simplex); antibacterial; antioxidant

Mechanisms of Action of Clove/Eugenol in Oral Care

A. Dental Analgesia (Toothache Relief):

- **TRPV1 channel blockade:** Eugenol initially activates then rapidly desensitizes TRPV1 (Transient Receptor Potential Vanilloid 1) pain receptors in dental pulp → analgesic effect
- **Voltage-gated sodium channel blockade:** Eugenol blocks Na+ channels in sensory nerve endings → prevents action potential generation → pain signal blocked (similar mechanism to local anesthetics)
- **Clinical use:** Eugenol used in Zinc Oxide Eugenol (ZOE) cement — temporary dental filling material; also used in endodontic sealers

B. Antibacterial:

- **Cell membrane disruption:** Eugenol disrupts bacterial cell membrane integrity → leakage of intracellular contents → bactericidal

- **Enzyme inhibition:** Inhibits ATPase enzyme, histidine decarboxylase, and fatty acid synthesis enzymes in oral bacteria
- **Spectrum:** Active against S. mutans, S. sanguinis, P. gingivalis, A. actinomycetemcomitans (key periodontal pathogen), Candida albicans

C. Anti-inflammatory:

- **COX and LOX inhibition:** Eugenol inhibits both cyclooxygenase (COX-1 and COX-2) and lipoxygenase → reduces prostaglandins and leukotrienes → anti-inflammatory
- **NF-kB inhibition:** Beta-caryophyllene activates CB2 receptors → inhibits NF-kB → reduces inflammatory cytokines (IL-1, IL-6, TNF-alpha)

D. Antioxidant:

- **ORAC value:** Clove has one of the highest antioxidant capacity of all spices (ORAC = 290,283 umol TE/100g)
- **Polyphenol activity:** Eugenol + flavonoids + tannins together provide powerful free radical scavenging — protect gum tissue from oxidative damage

Applications of Clove in Oral Care Products

Product	Clove Content	Benefit
Clove oil (direct application)	Pure clove EO (diluted 1:1 in carrier)	Emergency toothache relief; dental analgesic; antiseptic application to socket/pulp
Herbal toothpaste	0.1-0.5% clove oil or 0.5-2% clove extract	Antibacterial; anti-gingivitis; pleasant spicy-warm flavor; fresh breath
Antibacterial mouthwash	0.05-0.1% eugenol; 0.1-0.5% clove extract	Anti-plaque; anti-gingivitis; anti-halitosis; antiseptic rinse
Dental cement (ZOE)	Eugenol as liquid component	Temporary filling; sedative to pulp; analgesic; antibacterial sealing
Clove tooth powder	5-20% dried clove powder	Traditional toothpowder; mechanical cleaning + antibacterial
Herbal gum gel	1-3% clove extract	Applied to gums; analgesic; anti-gingivitis; soothing for teething pain
Listerine-type mouthwash	Methyl salicylate component	Clove oil is included in essential oil mouthwash blends with thymol and eucalyptol

Advantages and Disadvantages of Clove in Oral Care

Advantages	Disadvantages

Fastest acting natural dental analgesic — immediate toothache relief via TRPV1 and Na+ channel blockade	Eugenol can cause chemical burns and sensitization if used undiluted or excessively
Highest antioxidant capacity of any plant-based oral ingredient	Clove oil is cytotoxic to pulp cells at high concentrations — must be carefully diluted
Broad-spectrum antibacterial against caries, gingivitis, and Candida pathogens	Eugenol can interfere with composite resin polymerization — cannot be used as base under composite restorations
Professional dentistry use (ZOE cement) validates safety and efficacy	Strong, overpowering flavor — not preferred by all; limits consumer acceptance in modern toothpastes
Multi-mechanism: analgesic + antibacterial + anti-inflammatory + antioxidant	Risk of eugenol contact allergy (eugenol is a known sensitizer in the flavor/fragrance category)
Validated in Listerine mouthwash formula alongside thymol, menthol, eucalyptol	Standardization varies widely between commercial clove extracts; eugenol content must be specified

★ EXAM IMPORTANT POINTS

Q: Primary active in aloe vera? Acemannan (polysaccharide) — moisturization, wound healing, immunomodulation

Q: Primary active in turmeric? Curcumin — anti-inflammatory (NF-kB inhibition), tyrosinase inhibition, antioxidant

Q: Primary active in henna? Lawsone (2-hydroxy-1,4-naphthoquinone) — binds keratin; orange-red color

Q: Primary active in amla? Emblicanin A and B (stable Vit C); Ellagic acid — 5-AR inhibitor; anti-graying

Q: Primary active in neem? Nimbolide / Azadirachtin — antibacterial against S. mutans; anti-gingivitis

Q: Primary active in clove? Eugenol (72-90%) — dental analgesic via TRPV1/Na+ channel blockade; antibacterial

Q: How does amla prevent hair loss? 5-alpha reductase inhibition → reduces DHT → prevents follicle miniaturization

Q: ZOE cement uses? Zinc Oxide + Eugenol — temporary filling; analgesic; antibacterial sealing

SECTION C: ANALYTICAL COSMETICS — BIS SPECIFICATIONS AND ANALYTICAL METHODS

Analytical cosmetics involves the application of standard analytical techniques to ensure the quality, safety, and efficacy of cosmetic products through specifications (physicochemical parameters) and validated test methods as defined by Bureau of Indian Standards (BIS) and other regulatory bodies.

BIS (Bureau of Indian Standards) — Overview:

BIS is the national body for standardization in India (under Ministry of Consumer Affairs)

BIS IS (Indian Standard) codes set quality specifications for cosmetics

Cosmetics must comply with IS specifications under Drugs and Cosmetics Act 1940 and Rules 1945

Key parameters: appearance, pH, viscosity, active content, preservative efficacy, microbial limits

IS codes relevant: IS 6608 (shampoo), IS 6383 (skin cream), IS 11013 (toothpaste)

ANALYTICAL METHODS FOR SHAMPOO

BIS Reference: IS 6608:2004 — Shampoo Specification

A. Physical and Appearance Tests:

Test	BIS Specification	Method / Procedure
Appearance	Clear, uniform, no visible particles (clear shampoos); homogeneous (opaque)	Visual inspection in glass container against white and black backgrounds; examine for phase separation, haziness, particles
Color	As declared by manufacturer	Visual comparison with color standard; colorimeter (CIE L*a*b* system) for instrumental measurement
Odor	Characteristic, as declared	Organoleptic assessment by trained panel; no rancid, off, or foreign odor
Clarity (clear shampoos)	Clear without turbidity	Measured by UV-Vis spectrophotometer at 600 nm; Transmittance >90% at 600 nm for 'clear' claim

B. pH Determination:

Parameter	BIS Spec.	Method
pH	5.0-8.0 (recommended 5.5-7.5 for most shampoos)	10% w/v dilution of shampoo in distilled water; measure at 25°C using calibrated pH meter; 3 readings, average reported

- **Significance:** pH affects scalp health (acidic mantle 4.5-5.5), surfactant activity, preservative efficacy, and thickener performance (Carbomer requires pH >6)

C. Viscosity:

Parameter	BIS Spec.	Method
Viscosity	Typically 2000-30,000 cP depending on product type	Brookfield Viscometer (spindle 4-7) at 25°C; RV model; spindle speed: 10-50 rpm; report in cP (centipoise) or mPas

- **Significance:** Controls dispensing behavior, consumer perception, and stability. Too low = runny, poor foam; too high = difficult to dispense

D. Total Solids / Solids Content:

- **Method:** Accurately weigh 5g of shampoo in a pre-weighed petri dish; dry at 105°C for 4 hours in hot air oven; cool in desiccator; reweigh
- **Calculation:** Total Solids (%) = [(Weight after drying) / (Weight before drying)] x 100
- **BIS specification:** Minimum 10% total solids for most shampoos; declared value must be met

E. Active Matter (Surfactant) Content:

Parameter	BIS Spec.	Method
Total Active Matter (TAM)	Minimum 10-15% declared active	Two-Phase Titration (ASTM D1681): shampoo dissolved in water; acidified with H2SO4; titrate with BDH indicator solution with cetylpyridinium chloride (CPC) against indicator (mixed indicator of dimidium bromide + disulfine blue VI solution)

- **Two-Phase Titration Principle:** Anionic surfactant in aqueous phase + cationic titrant (benzethonium/tetradecyltrimethylammonium) form ion pairs that transfer into chloroform phase (lower phase) giving color change from pink to blue at endpoint

F. Foam Test (Foam Volume and Stability):

- **Method — Ross Miles Test:** 200 mL of 1% shampoo solution in distilled water at 40°C; pour 200 mL from a height of 90 cm into 200 mL solution already in cylinder; measure foam height immediately and after 5 minutes
- **Specification:** Initial foam height: minimum 80 mm; foam retention after 5 min: minimum 50% of initial height
- **Alternative: Cylinder shake test** — 10 mL of 1% solution in 100 mL graduated cylinder; shake 10 times; measure foam volume immediately and after 1 minute

G. Detergency Test (Soil Removal):

- **Method:** Prepare artificially soiled hair tresses (standardized artificial sebum applied); wash with 5% shampoo solution; measure soil removal by gravimetric analysis or UV-Vis spectrophotometry of wash solution
- **Specification:** Soil removal should be minimum 80% compared to blank control

H. Microbial Limit Test (IP / BIS IS 13001):

Test	BIS Specification	Method
Total Aerobic Microbial Count (TAMC)	Not more than 1000 CFU/mL	Membrane filtration or pour plate method; Soybean Casein Digest Agar (SCDA); 48-72 hours at 30-35°C
Total Yeasts and Molds (TYMC)	Not more than 100 CFU/mL	Sabouraud Dextrose Agar (SDA); 5-7 days at 20-25°C
Specified organisms	Absence of: P. aeruginosa, S. aureus, E. coli	Identification using MacConkey, Mannitol salt, Cetrimide agars; biochemical tests

I. Stability Testing of Shampoo:

- Accelerated stability: 45°C ± 2°C for 4 weeks; check phase separation, color, odor, pH, viscosity
- Freeze-thaw cycling: 3 cycles of -10°C to +25°C; 24 hours each condition; check for precipitation, separation
- Light stability: 200 watt·hour/m2 UV exposure; check for color change and photodegradation

J. Additional Tests for Conditioning Shampoo:

- **Silicone content:** GC-MS analysis of extracted silicone (after heptane extraction); quantify dimethicone content vs standard
- **Cationic polymer content:** Potentiometric titration with sodium dodecyl sulfate (SDS) vs cationic polymer; or HPLC with refractive index detector
- **Zinc content (anti-dandruff):** Atomic Absorption Spectroscopy (AAS) or ICP-OES for ZPT (zinc pyrithione) quantification

ANALYTICAL METHODS FOR SKIN CREAM

BIS Reference: IS 6383:1971 — Skin Cream Specifications

A. Physical Tests:

Test	BIS Specification	Method
Appearance	Smooth, uniform cream; no lumps or foreign matter	Visual and tactile examination; spread on glass plate; check uniformity and graininess under magnification
Color	White to off-white (unless tinted); uniform	Visual comparison; colorimetric measurement (CIE L*a*b*)
Odor	Characteristic; no rancid odor	Organoleptic assessment; trained panel evaluation
pH	4.5-7.5 (skin-compatible range)	10% w/v aqueous dispersion; calibrated pH meter at 25°C; 3 readings; report average
Consistency/Texture	Smooth, even spreadability	Penetrometer test: standard cone at 25°C; measure depth of penetration in 5 seconds; report in 1/10 mm units

B. pH Test for Skin Cream:

- **Preparation:** Dissolve/disperse 1g cream in 9 mL distilled water (10% w/v); warm gently if needed to disperse; cool to 25°C
- **Measurement:** Calibrate pH meter with pH 4.0 and pH 7.0 buffer solutions before measurement; measure three times; report average
- **Significance:** pH outside 4.5-7.5 can cause skin irritation; acidic creams (pH <5) may cause stinging in sensitive skin; alkaline creams may disrupt acid mantle

C. Water Content (Karl Fischer Titration):

- **Method:** Karl Fischer Titration (IP/USP method); accurately weigh 0.5-1.0g cream in titration vessel; titrate with Karl Fischer reagent to potentiometric endpoint
- **Karl Fischer Reagent:** Iodine + SO2 + base (pyridine/imidazole) + methanol; reaction: I2 + SO2 + H2O + base → products; each mole I2 reacts with 1 mole H2O
- **Significance:** Water content determines emulsion type (high water = O/W); affects microbial stability; W/O creams typically 20-40% water; O/W creams 60-80%

D. Emulsion Type Determination:

Test	O/W Emulsion Result	W/O Emulsion Result
Dilution test	Dilutes freely with water; stable dilution	Does NOT dilute with water; separates on water addition

Dye test (water-soluble dye — amaranth)	Continuous phase stains red uniformly (water = external)	Only droplets stain red; external phase colorless (oil = external)
Dye test (oil-soluble dye — Sudan Red)	Only droplets stain red; external phase colorless	Continuous phase stains uniformly red (oil = external)
Conductivity test	Good electrical conductivity (water conducts)	Poor electrical conductivity (oil does not conduct)
Filter paper test	Spreads readily on filter paper leaving wet mark	Does not spread readily; leaves no water mark

E. Viscosity of Skin Cream:

- **Method:** Brookfield Helipath Viscometer (for semi-solids); Spindle T-bar B or C; measure at 25°C; start at lowest speed (0.5 rpm) and increase; report viscosity at consistent shear rate
- **Alternative:** Cone and plate rheometer; measures true rheological behavior (shear thinning, yield stress) of cream
- **Specification:** Creams: 10,000-200,000 cP; lotions: 500-10,000 cP (depending on product design)

F. Spreadability Test:

- **Apparatus:** Glass plates (Lennard's apparatus); two glass plates (10x10 cm); 1g of cream placed between plates; weight of 200g placed on top plate for 1 minute at 25°C
- **Measurement:** Measure diameter (mm) of spread in 4 directions; calculate average spread area (pi x r2)
- **Specification:** Good spreadability cream: diameter 60-90 mm under standard conditions; very thick ointments may show less spread

G. Oil Content Determination (Soxhlet Extraction):

- **Method:** Weigh 5g cream in pre-weighed Soxhlet thimble; extract with petroleum ether (60-80°C) for 4-6 hours; evaporate solvent; weigh oil residue
- **Calculation:** Oil content (%) = [Weight of extracted oil / Weight of cream taken] x 100
- **Significance:** Verifies declared oil phase content; checks consistency between batches; oil:water ratio determines emulsion type

H. Microbial Limit Tests for Skin Cream:

Test	BIS/IP Specification	Method
Total Aerobic Microbial Count (TAMC)	Not more than 1000 CFU/g	Surface spread plate or pour plate; SCDA medium; 48-72h at 30-35°C

Total Yeasts and Molds	Not more than 100 CFU/g	SDA medium; 5-7 days at 20-25°C; inverted plates
Staphylococcus aureus	Absence per gram	Mannitol Salt Agar; coagulase test; catalase positive, coagulase positive
Pseudomonas aeruginosa	Absence per gram	Cetrimide agar; oxidase positive; fluorescent under UV

I. Challenge Test (Preservative Efficacy Test — PET / USP <51>):

- **Purpose:** Verify that preservative system prevents microbial growth in in-use contamination scenario
- **Test organisms:** S. aureus ATCC 6538, E. coli ATCC 8739, P. aeruginosa ATCC 9027, C. albicans ATCC 10231, Aspergillus brasiliensis ATCC 16404
- **Method:** Inoculate cream with each organism (10^5 - 10^6 CFU/g); incubate at 22-25°C; sample at day 0, 7, 14, 28; plate and count CFU
- **Acceptance criteria (Category A — skin creams):** Bacteria: 2-log reduction at day 7; no increase days 14-28; Fungi: no increase at days 14-28

J. Active Ingredient Assay (HPLC):

- **HPLC conditions:** C18 reverse-phase column; mobile phase: acetonitrile:water (varies by active); UV detection at appropriate wavelength; flow rate 1.0 mL/min
- **Sample preparation:** Extract active ingredient from cream matrix using appropriate solvent (methanol, acetonitrile); centrifuge; filter through 0.45 micron membrane
- **Calculation:** Compare peak area with external standard; calculate % of declared content; specification: 90-110% of labeled claim

ANALYTICAL METHODS FOR TOOTHPASTE

BIS Reference: IS 11013:1984 (revised) — Toothpaste Specification

A. Physical Tests:

Test	BIS Specification	Method
Appearance	Homogeneous, smooth paste; no separation of water or oil	Extrude from tube onto glass plate; examine for streaks, lumps, graininess, phase separation
Color	White or declared color; uniform throughout	Visual comparison against color standard; no mottling or streaks unless design feature
Consistency	Smooth, non-crumbling paste; good ribbon retention	Extrude 3 cm ribbon from tube onto glass plate; ribbon should retain shape for 30 seconds without slumping
pH	6.0-9.0 (typically 7.0-8.0 for fluoride toothpaste)	Slurry: 10% w/v dispersion of toothpaste in CO2-free water; measure at 25°C with calibrated pH meter
Moisture content	Maximum 35-45% depending on formulation	Karl Fischer titration; or gravimetric method: 2g paste at 105°C for 2h; moisture = weight loss

B. Fluoride Content Determination:

Fluoride is the most critical active in toothpaste for caries prevention. Accurate quantification is essential.

Method	BIS/IP Specification	Procedure Summary
Ion-Selective Electrode (ISE) — Primary method	900-1500 ppm F- (standard; adult toothpaste)	Slurry prepared in TISAB (Total Ionic Strength Adjustment Buffer); F- activity measured by fluoride ISE; compared with F- standard solutions; TISAB contains acetic acid, NaCl, citrate, CDTA
Fluoride total (soluble + insoluble)	Total F- = soluble + insoluble (from CaF2 precipitate)	Fusion or acid digestion method: fuse paste with NaOH; digest in H2SO4; measure total F- by ISE
Titrimetric method — SPADNS	Alternative colorimetric method	SPADNS reagent (sulfoAzo-III dye) + zirconyl chloride; fluoride displaces dye from Zr complex → bleaches color; measure absorbance at 570 nm; inversely proportional to F- concentration

- **TISAB (Total Ionic Strength Adjustment Buffer):** Contains acetic acid (58 mL), NaCl (58g), sodium citrate (4g), CDTA (cyclohexane diamine tetraacetic acid 0.3g) per liter; pH adjusted to 5.0-5.5 with NaOH; masks interfering ions; maintains constant ionic strength for accurate ISE reading

C. Abrasivity — RDA (Radioactive Dentin Abrasion) Test:

- **Purpose:** Measure the potential of toothpaste abrasives to abrade dentin — critical for safety; ensures toothpaste does not erode teeth with normal use
- **Method:** Standardized human tooth sections irradiated with neutrons (makes Ca radioactive); brush with toothpaste using standard brushing machine; measure radioactivity (Cs-134 or Ca-45) in abrasion slurry
- **RDA Values and Classification:**
  - RDA <70 = Low abrasion (sensitive teeth toothpaste; daily use for sensitive patients)
  - RDA 70-100 = Medium abrasion (standard toothpaste; FDA/ADA guideline: max 250 RDA for safe use)
  - RDA 100-150 = Relatively high (whitening toothpaste)
  - RDA >150 = High abrasion (professional whitening; not for daily use)
- **BIS specification:** Maximum RDA 250 for general toothpaste; sensitive teeth toothpaste should be <70 RDA

D. Foaming Test for Toothpaste:

- **Method:** Disperse 5g toothpaste in 100 mL warm distilled water (40°C) with stirring; transfer to 500 mL graduated cylinder; seal and shake 30 times; measure foam volume immediately and after 5 minutes
- **Specification:** Foam volume: minimum 150 mL in 500 mL cylinder; foam retention (5 min): minimum 70% of initial foam
- **Significance:** Foam helps distribute toothpaste throughout mouth; enhances consumer perception of cleanliness; SLS (0.5-2%) is primary foaming agent in toothpaste

E. Cleaning Power / Soil Removal:

- **Pellicle Cleaning Ratio (PCR) Test:** Measures removal of protein pellicle (stain) from tooth surface by toothpaste abrasive system
- **Method:** Bovine teeth coated with standardized artificial stain (ferrous gluconate or black tea); brushed with toothpaste using standard brushing machine; stain removal measured by reflectometry (CIE L* change)
- **Specification:** PCR minimum 70 (on scale of 100) for standard toothpaste; whitening toothpaste PCR 100-125

F. Heavy Metals (Lead, Arsenic, Mercury) — Safety Tests:

Heavy Metal	BIS Limit (IS 11013)	Method
Lead (Pb)	Not more than 20 ppm	Atomic Absorption Spectroscopy (AAS) at 217 nm; wet digestion of

		sample with HNO3 + H2SO4; aspirate into flame-AAS
Arsenic (As)	Not more than 5 ppm	Hydride Generation AAS (HGAAS); or ICP-OES; reduction with NaBH4 to arsine gas
Mercury (Hg)	Not more than 1 ppm	Cold Vapor AAS (CVAAS); reduction of Hg2+ with SnCl2; mercury vapor detected at 253.7 nm

G. Microbial Limit Tests for Toothpaste:

Test	BIS Specification	Method
Total Aerobic Microbial Count	Not more than 100 CFU/g	Membrane filtration or pour plate; SCDA; 30-35°C; 48-72 hours
Total Yeasts and Molds	Not more than 10 CFU/g	SDA medium; 20-25°C; 5-7 days
Staphylococcus aureus	Absent per gram	Mannitol salt agar; coagulase test
Pseudomonas aeruginosa	Absent per gram	Cetrimide agar; oxidase positive; pyocyanin production
Salmonella species	Absent per gram	XLD agar; Bismuth Sulfite Agar; biochemical confirmation

H. Preservative Assay (Methylparaben / Propylparaben):

- **HPLC Method:** Sample extraction in methanol; C18 reverse-phase column; mobile phase: acetonitrile:water:acetic acid (40:59:1); UV detection at 254 nm; compare with external standard
- **Specification:** Methylparaben 0.1-0.2%; Propylparaben 0.02-0.1%; Total paraben mix must not exceed regulatory limits

I. Triclosan Assay (in antibacterial toothpastes):

- **Method:** GC-MS analysis; extract with ethyl acetate; derivatize if needed; quantify vs triclosan reference standard; MRM mode for specificity in GC-MS
- **HPLC alternative:** Reverse-phase C18; mobile phase acetonitrile:water:formic acid; UV at 280 nm
- **Specification:** 0.3% ± 10% (i.e., 0.27-0.33% permitted range)

J. Stability Testing of Toothpaste:

- Accelerated stability: 45°C for 4 weeks; check appearance, pH, fluoride content, viscosity, tube integrity
- Syneresis test: store inverted tube at 40°C for 7 days; check for liquid separation from paste
- Freeze-thaw: 3 cycles -10°C to +25°C; check for crumbling, phase separation, consistency change
- Tube compatibility: assess interaction of paste with tube material (aluminum, plastic); migration of components into tube wall

★ EXAM IMPORTANT POINTS

Q: BIS IS code for shampoo? IS 6608:2004

Q: BIS IS code for skin cream? IS 6383:1971

Q: BIS IS code for toothpaste? IS 11013:1984

Q: What is RDA test? Radioactive Dentin Abrasion — measures toothpaste abrasivity; max RDA 250 (BIS)

Q: How is fluoride content in toothpaste measured? Ion-Selective Electrode (ISE) method in TISAB buffer; or SPADNS colorimetric method

Q: What is TISAB? Total Ionic Strength Adjustment Buffer — used with fluoride ISE; masks interfering ions

Q: SPF standard application dose for testing? 2 mg/cm2 (ISO 24444:2019)

Q: Two-phase titration is used to determine? Total Active Matter (surfactant content) in shampoo

Q: Emulsion type is determined by? Dilution test + dye tests (water-soluble and oil-soluble dyes) + conductivity test

Q: Preservative efficacy test organisms? S. aureus, E. coli, P. aeruginosa, C. albicans, Aspergillus brasiliensis

Q: Karl Fischer titration measures? Water content in skin cream

Q: What is Ross-Miles test? Foam height test for shampoo; pour 200 mL from 90 cm height; measure foam