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RESEARCH AND REVIEWS IN

CHEMICAL, BIOLOGICAL AND PHARMACEUTICAL SCIENCE

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PREFACE

The rapid expansion of knowledge in chemical, biological, and pharmaceutical sciences has profoundly transformed the way we understand nature, disease, and therapeutic innovation. *Research and Reviews in Chemical, Biological and Pharmaceutical Science* is conceived as a comprehensive scholarly platform that brings together contemporary research findings, critical reviews, and emerging perspectives across these interconnected disciplines. The book aims to bridge fundamental science with applied research, encouraging interdisciplinary dialogue that is essential for addressing complex scientific and societal challenges.

This volume presents carefully curated chapters contributed by researchers, academicians, and professionals who offer insights into recent advances in chemical synthesis, analytical techniques, biomolecular sciences, pharmacology, drug discovery, and healthcare technologies. Emphasis has been placed on review articles and research-based contributions that not only summarize current knowledge but also critically analyze trends, methodologies, and future directions. Such an approach enables readers to gain a holistic understanding of ongoing developments while identifying gaps that warrant further investigation.

In an era marked by rapid technological progress, integration of chemistry, biology, and pharmaceutical sciences has become indispensable. Innovations in areas such as medicinal chemistry, biotechnology, nanoscience, and translational research continue to redefine therapeutic strategies and improve quality of life. This book highlights these synergies, showcasing how collaborative research can lead to sustainable solutions in medicine, industry, and environmental management.

Research and Reviews in Chemical, Biological and Pharmaceutical Science is intended to serve as a valuable reference for undergraduate and postgraduate students, research scholars, faculty members, and professionals in academia and industry. The editors sincerely hope that this volume will inspire scientific curiosity, promote rigorous research, and contribute meaningfully to the advancement of knowledge in chemical, biological, and pharmaceutical sciences.

- Editors

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GREEN CHEMISTRY APPROACHES FOR SUSTAINABLE CHEMICAL PROCESSES

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Abstract:

Green chemistry has emerged as a transformative framework for redesigning chemical products and processes to minimize environmental impact, enhance resource efficiency, and promote sustainability across the chemical industry. By emphasizing waste prevention, safer chemicals, renewable feedstocks, energy efficiency, and benign reaction conditions, green chemistry provides practical solutions to global challenges such as pollution, climate change, and resource depletion. This chapter presents a comprehensive overview of green chemistry principles and their application in sustainable chemical processes. Key strategies including atom economy, alternative solvents, catalysis, renewable raw materials, process intensification, and life cycle assessment are discussed in detail. Industrial case studies and emerging trends such as nanotechnology-enabled green chemistry, biocatalysis, and digital process optimization are highlighted. The chapter concludes by addressing current challenges, regulatory perspectives, and future directions necessary for large-scale implementation of green chemistry in academia and industry.

Keywords: Green Chemistry, Sustainable Processes, Catalysis, Renewable Feedstocks, Atom Economy, Industrial Sustainability.

1. Introduction:

The chemical industry plays a central role in modern society, providing materials, energy carriers, pharmaceuticals, agrochemicals, and consumer products essential for economic development and quality of life. However, conventional chemical processes have historically relied on hazardous reagents, toxic solvents, non-renewable resources, and energy-intensive operations, leading to significant environmental pollution and health risks. Increasing awareness of climate change, ecosystem degradation, and human toxicity has intensified the demand for cleaner and more sustainable chemical technologies.

Green chemistry, formally articulated in the 1990s, offers a proactive and science-driven approach to sustainability by designing chemical products and processes that reduce or eliminate the use and generation of hazardous substances. Unlike traditional pollution control strategies that focus on remediation, green chemistry emphasizes prevention at the molecular level. The integration of green chemistry into chemical manufacturing aligns closely with the broader goals of sustainable development, circular economy, and responsible innovation.

This chapter aims to provide an in-depth discussion of green chemistry approaches for sustainable chemical processes. It outlines the fundamental principles of green chemistry, explores key technological strategies, and examines real-world industrial applications. Emphasis is placed on how green chemistry can simultaneously achieve environmental protection, economic viability, and social responsibility.

2. Principles of Green Chemistry

The principles of green chemistry provide a conceptual and practical foundation for designing chemical products and processes that minimize environmental impact while maintaining functionality and economic feasibility. Rather than serving as rigid rules, these twelve principles act as flexible guidelines that can be adapted across different branches of chemistry and scales of operation.

i. Prevention of Waste: The most effective way to reduce environmental harm is to prevent waste formation at its source. Green chemistry emphasizes designing processes that inherently generate little or no waste, rather than relying on treatment, cleanup, or disposal after waste has been produced.

ii. Atom Economy: Chemical reactions should be designed so that the maximum proportion of reactant atoms is incorporated into the final product. High atom economy reactions reduce by-product formation, conserve raw materials, and lower costs associated with waste management.

iii. Less Hazardous Chemical Syntheses: Whenever possible, synthetic methods should use and generate substances with minimal toxicity to humans and the environment. This principle encourages the replacement of hazardous reagents with safer alternatives without compromising reaction efficiency.

iv. Designing Safer Chemicals: Chemical products should be designed to perform their intended function while exhibiting reduced toxicity. This principle promotes molecular design strategies that minimize adverse health and ecological effects throughout a product's life cycle.

v. Safer Solvents and Auxiliaries: Solvents, separation agents, and other auxiliary substances should be eliminated where feasible or replaced with safer alternatives such as water, bio-based solvents, or solvent-free systems, thereby reducing environmental and occupational hazards.

vi. Energy Efficiency: Energy requirements should be minimized due to their economic and environmental impacts. Conducting reactions at ambient temperature and pressure, or using

alternative activation methods, significantly lowers energy consumption and associated emissions.

vii. Use of Renewable Feedstocks: Raw materials should be derived from renewable resources rather than depleting fossil reserves, whenever technically and economically viable. Biomass-based feedstocks contribute to long-term resource sustainability and reduced carbon footprints.

viii. Reduction of Derivatives: Unnecessary derivatization steps such as protection and deprotection should be avoided, as they require additional reagents and generate extra waste. Streamlined synthetic routes enhance efficiency and sustainability.

ix. Catalysis: Catalytic reagents are preferred over stoichiometric ones because they increase reaction selectivity, reduce energy demands, and minimize waste generation. Both chemical and biological catalysts play key roles in sustainable synthesis.

x. Design for Degradation: Chemical products should be designed to break down into non-toxic, environmentally benign substances after use, preventing accumulation and long-term persistence in the environment.

xi. Real-Time Analysis for Pollution Prevention: Analytical methods should allow real-time monitoring and control of chemical processes to prevent the formation of hazardous substances. Early detection enables corrective action before pollution occurs.

xii. Inherently Safer Chemistry for Accident Prevention: Substances and process conditions should be chosen to minimize the potential for chemical accidents, including fires, explosions, and toxic releases. Designing safer processes enhances both environmental protection and worker safety.

Together, these principles encourage a holistic approach to chemical innovation, integrating environmental responsibility, process efficiency, and safety at every stage of chemical research and industrial manufacturing.

3. Atom Economy and Waste Minimization

Atom economy is a quantitative measure of how efficiently reactants are incorporated into the final product. High atom economy reactions generate minimal by-products and reduce waste disposal costs. Modern synthetic strategies such as addition reactions, rearrangements, and multicomponent reactions exhibit superior atom economy compared to traditional substitution or elimination reactions.

3.1 Examples of Atom-Efficient Reactions

The concept of atom economy is best illustrated through specific chemical reactions:

i. Diels–Alder Cycloaddition Reaction: This [4+2] cycloaddition reaction combines a diene and a dienophile to form a six-membered ring without generating by-products. Nearly all atoms of the reactants are incorporated into the product, resulting in close to 100% atom economy. This reaction is widely used in pharmaceutical and polymer synthesis.

ii. Hydrogenation of Alkenes: In catalytic hydrogenation, hydrogen adds directly across a carbon–carbon double bond in the presence of a metal catalyst (e.g., Pd, Ni). Since no atoms are discarded, the reaction demonstrates excellent atom economy and is extensively applied in fine chemical and food industries.

iii. Multicomponent Reactions (MCRs): Reactions such as the Ugi and Passerini reactions involve three or more reactants forming a single product in one step. These reactions maximize atom utilization while minimizing solvent use and purification steps, making them highly attractive for sustainable synthesis.

iv. Olefin Metathesis: Olefin metathesis rearranges carbon–carbon double bonds using metal-carbene catalysts. This catalytic process efficiently redistributes atoms between molecules with minimal waste formation and has revolutionized industrial polymer and pharmaceutical synthesis.

v. Rearrangement Reactions: Reactions such as the Beckmann and Claisen rearrangements reorganize atoms within a molecule without introducing additional reagents, thereby maintaining high atom economy.

Process intensification techniques, including one-pot syntheses and telescoped reactions, further contribute to waste minimization by reducing solvent use, purification steps, and energy consumption. Metrics such as E-factor and process mass intensity (PMI) are widely used to assess and benchmark the environmental performance of chemical processes.

3.2 Numerical Example of Atom Economy Calculation

Atom economy is calculated using the following expression (Trost, 1991):

Atom economy (%) = (Molecular weight of desired product / Sum of molecular weights of all reactants) × 100

Example: Hydrogenation of Ethene to Ethane

Reaction: Ethene (C_2H_4) + Hydrogen (H_2) → Ethane (C_2H_6)

Molecular weights:

- Ethene (C_2H_4) = 28 g·mol^{-1}
- Hydrogen (H_2) = 2 g·mol^{-1}
- Ethane (C_2H_6) = 30 g·mol^{-1}

Calculation: Atom economy = (30 / (28 + 2)) × 100 = (30 / 30) × 100 = **100%**

This example illustrates an ideal green reaction where all atoms of the reactants are incorporated into the final product, resulting in zero by-product formation.

Comparative Example: Williamson Ether Synthesis

Reaction (simplified): R–ONa + R′–Cl → R–O–R′ + NaCl

In this case, sodium chloride (NaCl) is formed as an unavoidable by-product. Since only part of the reactant mass contributes to the desired ether product, the atom economy is significantly

lower than 100%. This comparison highlights why addition and catalytic reactions are favored in green chemistry over substitution reactions that generate stoichiometric waste.

Table 1: Comparison of Conventional and Green Chemical Processes

Parameter	Conventional Chemical Processes	Green Chemical Processes
Raw materials	Fossil-based, non-renewable	Renewable and bio-based feedstocks
Solvents	Volatile organic solvents	Water, bio-solvents, solvent-free
Energy use	High temperature and pressure	Ambient or low-energy conditions
Waste generation	High (by-products, effluents)	Minimal waste, high atom economy
Catalysis	Stoichiometric reagents	Recyclable homogeneous / heterogeneous catalysts
Environmental impact	High toxicity and emissions	Reduced toxicity and emissions

4. Green Solvents and Reaction Media

Solvents account for a significant fraction of waste and toxicity in chemical processes. The replacement of volatile organic solvents with environmentally benign alternatives is a key strategy in green chemistry. Promising green solvents include:

- **Water**, as a non-toxic and abundant solvent.
- **Supercritical fluids**, particularly supercritical CO_2.
- **Ionic liquids** with tunable properties.
- **Deep eutectic solvents (DESs)** derived from biodegradable components.
- **Bio-based solvents** such as ethanol, ethyl lactate, and glycerol derivatives.

Solvent-free reactions and solid-state chemistry further reduce environmental burden by eliminating solvents altogether.

5. Catalysis for Sustainable Processes

Catalysis is a cornerstone of green chemistry, enabling reactions to proceed with higher selectivity, lower energy input, and reduced waste. Both homogeneous and heterogeneous catalysts play crucial roles in sustainable chemical processes.

5.1 Homogeneous Catalysis

Transition metal complexes and organocatalysts facilitate highly selective transformations under mild conditions. Advances in ligand design have improved catalyst stability and recyclability, significantly lowering solvent and energy requirements.

5.2 Heterogeneous Catalysis

Solid catalysts offer advantages in ease of separation and reuse. Zeolites, metal oxides, and supported metal nanoparticles are widely used in petrochemical and fine chemical industries due to their durability and scalability.

5.3 Biocatalysis

Enzymes provide exceptional selectivity and operate under environmentally benign conditions such as aqueous media and ambient temperature. Biocatalytic processes are increasingly adopted in pharmaceutical and agrochemical synthesis.

Table 2: Types of Catalysts Used in Green Chemistry

Catalyst type	Key features	Typical applications
Homogeneous catalysts	High selectivity, tunable	Fine chemicals, pharmaceuticals
Heterogeneous catalysts	Reusable, easy separation	Petrochemicals, bulk chemicals
Biocatalysts	Highly specific, mild conditions	Drug synthesis, agrochemicals

6. Renewable Feedstocks and Bio-based Chemistry

The transition from fossil-based raw materials to renewable feedstocks is essential for long-term sustainability. Biomass-derived resources such as carbohydrates, lignin, vegetable oils, and bio-waste serve as precursors for fuels, polymers, and specialty chemicals.

Green chemistry enables efficient biomass conversion through catalytic, enzymatic, and thermochemical pathways. Bio-refineries integrate multiple processes to maximize value extraction from renewable resources.

7. Energy-Efficient and Alternative Activation Methods

Reducing energy consumption is critical for sustainable chemical manufacturing. Alternative activation techniques include:

- **Microwave-assisted synthesis** for rapid heating.
- **Ultrasound (sonochemistry)** to enhance reaction rates.
- **Photocatalysis** utilizing visible or solar light.
- **Electrochemical synthesis** as a reagent-free redox approach.

These methods often enable reactions at lower temperatures and shorter times, contributing to reduced carbon footprints.

8. Industrial Applications and Case Studies

Green chemistry principles have been successfully implemented across a wide range of industrial and societal sectors, demonstrating their versatility and practical relevance beyond traditional chemical manufacturing.

8.1 Pharmaceutical and Fine Chemical Industries

In the pharmaceutical sector, green chemistry has led to the development of safer and more efficient routes for active pharmaceutical ingredients (APIs). The use of catalytic asymmetric synthesis, biocatalysis, and solvent minimization has significantly reduced waste and improved process safety. Notable examples include greener synthesis routes for ibuprofen and atorvastatin, which reduced the number of synthetic steps and eliminated hazardous reagents.

8.2 Polymer and Materials Science Applications

Green chemistry has enabled the production of biodegradable, bio-based, and recyclable polymers such as polylactic acid (PLA), polyhydroxyalkanoates (PHAs), and bio-based polyethylene. Sustainable polymerization techniques, including solvent-free and catalytic processes, contribute to reduced carbon footprints and plastic waste mitigation.

8.3 Agrochemicals and Crop Protection

In agrochemical manufacturing, green chemistry supports the synthesis of safer pesticides and fertilizers with reduced toxicity and improved efficiency. Controlled-release formulations, bio-pesticides, and greener synthesis pathways minimize environmental contamination and enhance agricultural sustainability.

8.4 Energy and Fuel Technologies

Green chemistry plays a vital role in sustainable energy technologies, including biofuels, hydrogen production, and battery materials. Catalytic conversion of biomass to liquid fuels, green hydrogen generation via electrolysis, and environmentally benign electrode materials exemplify its contribution to clean energy transitions.

8.5 Environmental Remediation and Pollution Control

Green chemistry-based materials and processes are widely applied in environmental remediation. Photocatalysts, adsorbents, and bio-based flocculants are used for water purification, wastewater treatment, and air pollution control, offering efficient and eco-friendly alternatives to conventional methods.

8.6 Consumer Products and Household Chemicals

The formulation of eco-friendly detergents, cleaners, paints, and coatings has been significantly influenced by green chemistry. The replacement of toxic surfactants, solvents, and additives with biodegradable alternatives improves human safety and reduces environmental persistence.

8.7 Electronics and Advanced Manufacturing

In the electronics industry, green chemistry supports lead-free soldering materials, safer etching processes, and sustainable semiconductor fabrication. These innovations reduce hazardous waste generation and occupational exposure risks.

These diverse applications demonstrate that green chemistry can enhance both environmental and economic performance while supporting innovation across multiple industrial sectors.

9. Life Cycle Assessment and Sustainability Metrics

Life cycle assessment (LCA) is a systematic tool used to evaluate the environmental impact of chemical products and processes from raw material extraction to end-of-life. Integrating LCA with green chemistry design ensures informed decision-making and avoids burden shifting.

Complementary sustainability metrics, including carbon intensity, water footprint, and toxicity indices, support comprehensive process evaluation.

10. Challenges and Future Perspectives

Despite significant progress, several challenges hinder widespread adoption of green chemistry. These include high initial costs, scalability issues, limited availability of renewable feedstocks, and regulatory barriers. Education, interdisciplinary collaboration, and supportive policy frameworks are essential for overcoming these obstacles.

Future developments are expected to focus on digitalization, artificial intelligence-assisted process design, nanotechnology-enabled catalysis, and circular chemistry concepts. Continued innovation will be crucial for achieving truly sustainable chemical industries.

Conclusion:

The analysis presented in this chapter underscores the role of green chemistry as a central driver for advancing sustainability within chemical science and industry. By focusing on preventive design strategies—such as atom-efficient reactions, judicious solvent selection, catalytic transformations, renewable raw materials, and energy-conscious methodologies—green chemistry offers practical solutions to many of the environmental challenges traditionally associated with chemical manufacturing.

The breadth of applications discussed illustrates that green chemistry is not confined to a single sector but is broadly relevant to pharmaceuticals, materials, agriculture, energy systems, environmental protection technologies, and advanced manufacturing. The incorporation of quantitative evaluation tools, including atom economy, E-factor analysis, and life cycle assessment, further strengthens the ability to compare alternative processes and make evidence-based sustainability decisions.

Although economic, technical, and regulatory challenges remain, ongoing progress in catalysis, biocatalysis, digital process optimization, and circular chemistry continues to expand the feasibility of greener alternatives. Ultimately, embedding sustainability considerations at the molecular and process-design levels will be essential for developing resilient, competitive, and socially responsible chemical industries capable of meeting long-term global sustainability objectives.

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GREEN SYNTHESIS OF TITANIUM OXIDE NANOPARTICLES FROM THE AQUEOUS EXTRACT OF *ABUTILON INDICUM* LEAVES AND THEIR ANTI-DIABETIC ACTIVITY

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Abstract:

Titanium oxide nanoparticles (TiONPs) synthesized from plant sources are eco-friendly, cost effective and have a wide range of applications. The antidiabetic property of titanium dioxide produced by various herbal sources has well been studied. *Abutilon indicum* leaves have been used in this study to synthesis TiONPs due to their antidiabetic properties. This current investigation was to examine titanium oxisde nanoparticles were efficiently green synthesized by using *Abutilon indicum* leaves was extracted with aqueous solution. The colour change from white precipitate to pale yellow indicates the synthesis of titanium oxide nanoparticles (TiONPs). The TiONPs was characterized by UV spectrophotometer the range at 314 nm. The functional group was analyzed by FTIR. The particle size was analyzed by X-ray diffraction (XRD). The SEM analysis was to evaluate the morphology and size of synthesized nanoparticles. TiONPs are showed the presence of hexagon shape and the particle size ranged from 62.8 nm to 124 nm. EDAX analysis of used for synthesized titanium oxide nanoparticles. The spectrum confirmed the presence of a strong peak for elemental Titanium at approximately 4.5 keV. Antidiabetic activity analysis was performed by α-amylase activity and α-glucosidase activity. The percentage inhibition of α-amylase was exhibited at 80.9% in TiONPs and 81.9% in Acarbose (standard). The percentage inhibition of α-glucosidase was exhibited at 78.5% in TiONPs and 84.6% in Acarbose (standard). The finding results revealed that the green synthesized TiONPs showed moderate antidiabetic activity.

Keywords: Titanium Oxide, *Abutilon indicum,* SEM, Antidiabetic

Introduction:

Abutilon indicum is known as “Atibala” in Sanskrit. Literally “Ati” means very and “Bala” means powerful, referring to the properties of this plant as very powerful. *Abutilon indicum* is a hairy herb or under shrub distributed throughout the tropica. In traditional systems of medicine, various plant parts such as roots, leaves, flowers, bark, seeds, and stems have been used as

antioxidant, demulcent, laxative, diuretic, analgesic, anti-inflammatory and antiulcer agents. The leaves are reported to be used by traditional practitioners in cases of inflammatory joint disorders as folklore remedy. Psychosomatic medicinal use related to scorpion bite treatment is also reported in India. Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties. It contributes the beneficial effect on health or plays an active role in interest in bioactive secondary metabolites of plant amelioration of disease. The medicinal properties of the plants have been investigated in the recent scientific developments throughout the world, due to their potential antioxidant activity, no side effects and economic viability. Antioxidant property of the plant is mainly due to the presence of phenolic compounds. The importance of medicinal plants and the contribution of phytomedicine to the well- being of a significant number of the world's population have attracted interest from a variety of disciplines. The present review is therefore an effort to give the detailed survey of literature on its Pharamacognosy, phytochemistry as well as traditional and pharmacological uses.

The various parts of the plant *Abutilon indicum* (L.) Sweet such as leaves, roots, flowers, seeds and seed oil are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments. The plant has been a reputed remedy in the Siddha systems of medicine for piles, jaundice, leprosy and ulcer (Yoganarasimhan *et al.,* 2002). In Vedic periods, the roots of the Bala plants i.e. Atibala (*A. indicum* Linn.), Mahabala (*Sida rhombifolia* Linn.), Bala (*Sida cordifolia* Linn.) and Bhumibala (*Sida veronicaefolia* Lam) were used to remove poison, vata–pitta diseases, heart problems, bily blood, eye diseases and uterine disorders. Its seeds and roots both were used in fever in the form of decoction (Dwivedi, 2000). Following various folk claims for cure of numerous diseases, efforts have been made by researchers to verify the efficacy of the plant through scientific biological screenings. The plant contains saponins, flavonoids, alkaloids, hexoses, n-alkane mixtures (C22-34), alkanols, and amino acids as main classes of compounds.

Leaves: Plant leaves are demulcent; given as decoction for bronchitis, bilious diarrohea, gonorrhea, bladder inflammation, urethritis and fevers. It is also used as eyewash, mouthwash, in toothache and tender gums; gonorrhoea, quick ulcer healing and inflammation of the bladder. Its extract is also used in relieving thirst and in reducing fever. The leaf juice mixed with jaggery is used for the treatment of snakebite as antidote (Mohapatra *et al.,* 2008). The bread prepared from the mixture of leaf powder and wheat flour is taken daily during night for about one month for cure of uterus displacement (Ganeshan, 2007). Flowers: The flowers are used to increase semen in men. Flower paste is applied to boils and ulcers. Seeds: Seeds are demulcent, laxative, expectorant and aphrodisiac; useful for gonorrhea and cystitis. In China it is used for tinnitus, deafness, earaches, fevers, hives, tuberculosis, weeping ulcers and as diuretic. In India, seeds are used for coughs and fevers, bronchitis, dysuria, diabetes, dysmenorrhea, diarrhea, boils and skin

ulcers. The seeds from this plant are used in the treatment of cough, puerperal disease, urinary disorders, chronic dysentery and fever (Jayaweera, 1982). Roots: Infusion of root is useful in fever as a cooling medicine, stranguary, heamaturia. Root is used as a pulmonary sedative and diuretic and can be taken for the relief of hematuria. It is also effective in the treatment of leprosy. Roots and bark are used as aphrodisiac, anti- diabetic and nervine tonic. Stem bark: The bark is used as a diuretic, anthelmintic, pulmonary, sedative and also used in fever (Kahmiri MA *et al.,* 2013). Bark is astringent and is used in stranguary and urinary complaints. Fruit: Fruit decoction mixed with ammonium chloride is given orally to treat haemorrhagic septicemia (Ali ZA, *et al.,* 1999). The fruit is used to treat piles, gonorrhoea and cough (Samy *et al.,* 2008 & Ignacimuthu *et al.,* 2008). Whole plant: The folk practitioner also uses this plant for curing blood dysentery, fever, allergy and is also an aphrodisiac (Seetharam *et al.,* 2002). The whole plant is used as anti-inflammatory, immune stimulating effect and in piles. It has been reputed in the siddha system of medicine as a remedy for jaundice, piles, ulcer and leprosy (Yoganarasimhan *et al.,* 200). knowledge of individual chemical constituents of a medicinal plant is essential for understanding the pharmacological activity as well as potential toxicity and optimizing extraction procedures. A number of constituents have been reported from species of *A. indicum* and they belong to lactones, sesquiterpenes, flavanoid aglycones, steroids, carbohydrates, phenols, tannins, alkaloids, flavanoids glycosides, proteins, alkaline sulphates and amino acids (Patel *et al.,* 2011). Leaves: Leaves contain tannins, mucilage, traces of asparagin, organic acid and, ash of leaves contains alkaline sulphates, chlorides, magnesium phosphate and calcium carbonate (Panda *et al.,* 2000). According to Rajlakshmi, ethanolic extract contain 72% more quercetin than flowers (Rajalakshmi *et al.,* 2009). Leaves also contains alkaloids, sterols, titerpenoids, glycosides29 essential oils as well various amino acids. Baxi *et al.* (1980) isolated α- tocopherol and β–sitosterol from leaves.

This study reported the effectiveness of powdered dried aerial parts of *A. indicum* in decreasing the severity of commonly observed symptoms of bronchial asthma i.e. dyspnoea, cough, chest tightness and wheezing. It was also found to significantly increase the pulmonary function in patients having mild to moderate bronchial asthma (Paranjape *et al.,* 2006). Diuretic activity: Seed extract of *A. indicum* were evaluated for its diuretic effect wherein the aqueous extract at 400 mg/kg exhibited statistically significant effect when compared with reference standard Furosemide. Hence, study elucidated that extract posses significant diuretic and natriuretic effect but not potassium sparing effect (Balamurugan *et al.,* 2010). Immunomodulatory activity: Dashputre *et al.* studied the immunomodulatory activity of ethanolic and aqueous extract of *A. indicum* leaves. This activity was said to be attributed to the presence of flavonoids (quercetin), alkaloids, tannins, saponin glycosides and phenolic compounds.

Materials and Methods

Cold Extraction

10 gm of sample was weighed and soaked in 100 ml of sterile distilled water. The extract was allowed to stand 24h and filtered using sterile filter paper. The filtrate was collected and used for synthesis of titanium oxide nanoparticles.

Synthesis of Titanium Dioxide Nanoparticles (Thakur *et al.,* 2019; Mohammad Zaki Ahmad *et al.,* 2022)

10 ml of extract was added to 90 ml of 5 mM aqueous TiO2 solution and stirred on a magnetic stirrer at 500 rpm for 2 h at room temperature. This solution was incubated at room temperature for 6 h. After 6 hr centrifuged at 8000 rpm for 10 min. Collect the pellet and washed with distilled water. This content was dried at 60ºC for 1 h. This Titanium dioxide nanoparticles were stored for further analysis.

Characterization of Nanoparticles

UV–Visible Spectrophotometer:

UV–visible spectrophotometer was used to obtained spectral response of ZnONPs. The sample was monitored by absorbance measurements carried out on UV–visible spectrophotometer in the wavelength range of 200-800nm (Thermo Scientific—Evolution 201). It refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

Fourier Transforms Infrared Spectroscopy (FTIR):

Fourier-transform infrared spectroscopy (FTIR) analysis was used for the identifying the functional group of the nanoparticles. It was used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. It was analyzed by SHIMADZU spectrometer in the range of 500–4000 cm−1.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) is used to determine the atomic and molecular structure of nanoparticles. This method was carried out for irradiating of the material with incident X-rays and to measure the intensities and scattering angles of the X-rays which was scattered by the nano material. The X-ray beam is diffracted by the sample and detected at various angles. The

XRD (RIGAKU miniflex-600, Japan) was performed using an X-ray diffractometer–Cu, Kα radiation λ 1.54 nm in the 2θ range of 30-80 operated data voltage of 40kV and a current of 30 mA. The graph is detected between 2θ in x-axis and intensity on y-axis with different peaks corresponding to different planes of the crystal.

SEM/EDAX Analysis

Scanning electron microscopy (SEM) analyzed for the size and shape of the nanoparticles were examined. SEM provides detailed high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or backscattered electron signal. The images of titanium oxide nanoparticles were examined using scanning electron microscopy (SEM; TESCAN VEGA3 SBU).

Antidiabetic Activity

Inhibition of Alpha Amylase (Suhashini *et al.*, 2014 alpha amylase)

Procedure

Different concentrations of samples and standard drug were taken. Then 1 ml of α-amylase in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube and was incubated at 25°C for 30 min. Then 1 ml of 1% starch solution in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 3 min. The reaction was stopped with 1 ml of 3, 5 dinitrosalicylic acid. 9 ml of distilled water was added to the reaction mixture. Absorbance was measured at 540 nm.

$$\% \text{ of alpha amylase inhibition} = \frac{\text{OD sample} - \text{OD control}}{\text{OD sample}} \times 100$$

Inhibition of Alpha Glucosidase (Elsnoussi Ali Hussin Mohamed *et al.*, 2012 alpha glucosidase)

Procedure

100 µl of 0.1 U glucosidase was taken in different tubes. To this 50 µl of sample and standard of different concentrations were added (should not mix) and incubated at 25°C for 10 min. Then 50 µl of p-nitrophenyl alpha- D-glucosidase was added, vortexed and incubated at 25°C for 5 min. Add 800 µl of stop solution (0.1 M sodium carbonate) was added. Absorbance was measured at 405 nm.

$$\% \text{ of alpha glucosidase inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Results and Discussion:

The X-ray diffraction (XRD) pattern of synthesized Titanium oxide nanoparticles was showed at 25.25, 28.37, 36.92, 37.77, 38.54, 40.49, 48.00, 53.84 and etc., corresponding to the lattice

planes 630, 72.1, 88, 205, 89 and 30 nm are in accordance with the reported pattern (JCPDS 04-0783) which confirmed that the green synthesized TiONPs are nanocrystalline. Similarly, Shakeel Ahmad *et al.,* 2020 studied the X-ray diffraction (XRD) pattern of synthesized MnO nanoparticles using *Abutilon indicum* showing greater peak value at angle of 37˚ indexing to (101), (110), and (200) crystal planes at an angle of 37°, 56°, and 67°, respectively.

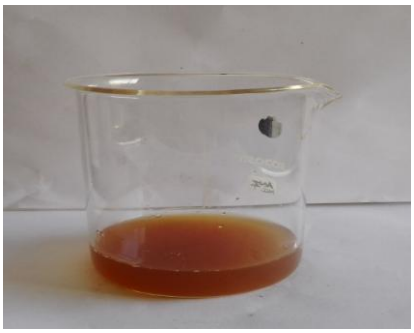
The SEM analysis was to evaluate the morphology and size of synthesized nanoparticles. SEM is most widely used technique for characterizing the nanoparticles in terms of the physical morphology of the particles. The capping of nanoparticles was preventing agglomeration of the particles and stabilizing in the medium. TiONPs are showed the presence of hexagon shape and the particle size ranged from 62.8 nm to 124 nm. EDAX of synthesized titanium oxide nanoparticles confirmed the presence of a strong peak for elemental Titanium at approximately 4.5 keV. SEM image of silver nanoparticle synthesized from *Abutilon indicum* is spherical shape with size of ranging from 1-100 nm reported by J. Uthaya Chandirika et al.

Anti-diabetic activity analysis was performed by alpha amylase and alpha glucosidase assays. The percentage inhibition of albumin denaturation was exhibited at 37.5% in TiONPs and 84.6% in diclofenac (standard) (Figure 8, Chart 1 and Table 3). The percentage inhibition of membrane stability was found to be 80% in ethanol extract and 53.6 % in standard diclofenac (Figure 9, Chart 2 and Table 4). The percentage inhibition of protein denaturation was exhibited at 81.2% in ethanol extract and 87.5 % in standard diclofenac (Figure 10, Chart3 and Tab. The finding results revealed that the green synthesized TiONPs showed moderate antiinflammatory activity.

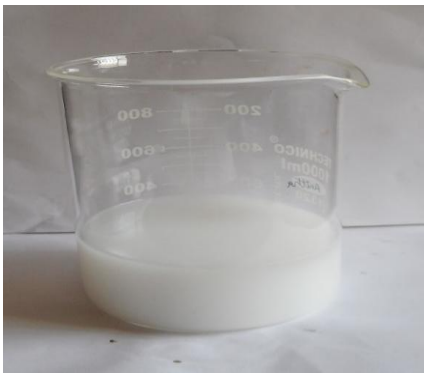
The inhibition of these enzymes lowers the blood glucose level in blood. The alpha-amylase and alpha-glucosidase were inhibited in a concentration-dependent manner followed by various concentrations of TiONps. The enzymatic activity was lowered remarkably when the concentration of TiONPs was increased. The percentage inhibition of amylase enzyme by standard and synthesized TiONPs was 81.8% and 80.9%. The percentage inhibition of glucosidase enzyme by standard and synthesized TiONPs was 84.6% and 78.5%. Seetharam studied the antidiabetic potential of synthesized nanoparticles from *Abutilon indicum* by oxidase-peroxidase method on administration of 400mg/kg.p.o. significantly reduced the blood glucose levels to extend of 23.10% & 26.95%. The antidiabetic studies results proved that TiONPs synthesized from aqueous extract of *Abutilon indicum* can be used to reduce blood glucose levels. It has notable applications in the pharmaceutical and biomedical industries.

Powdered leaves of *Abutilon indicum*

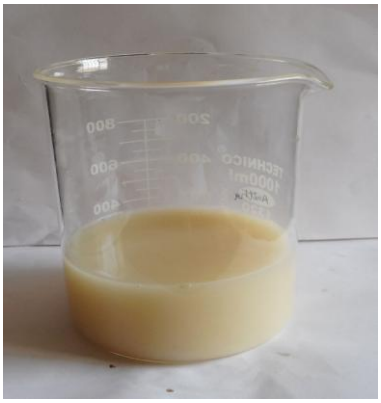
***Abutilon indicum* leaves extract**



***Abutilon indicum* filtrate**

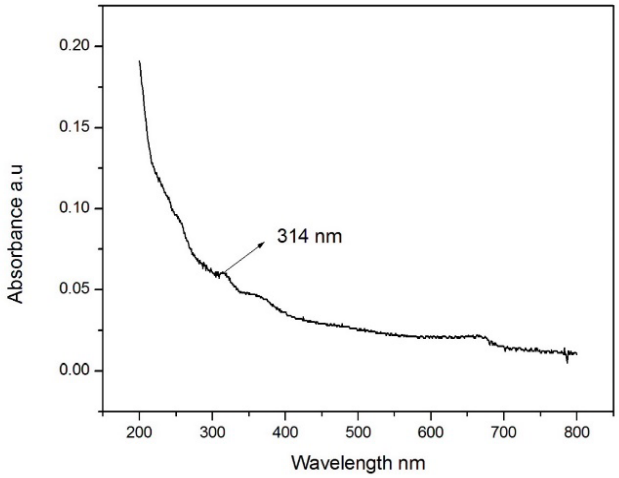


Synthesis of Titanium oxide nanoparticles using aqueous extract of *Abutilon indicum* leaves using Titanium solution

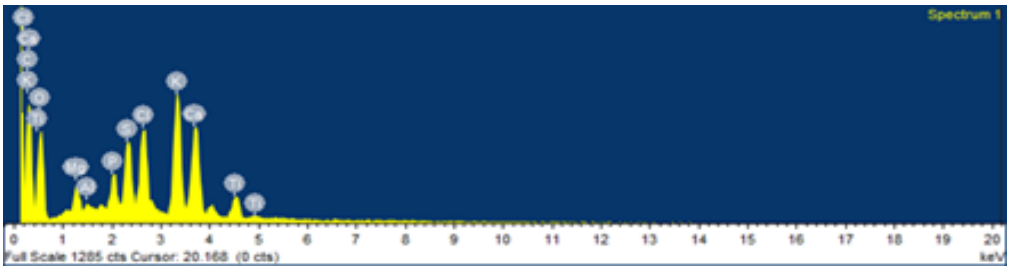


After synthesis

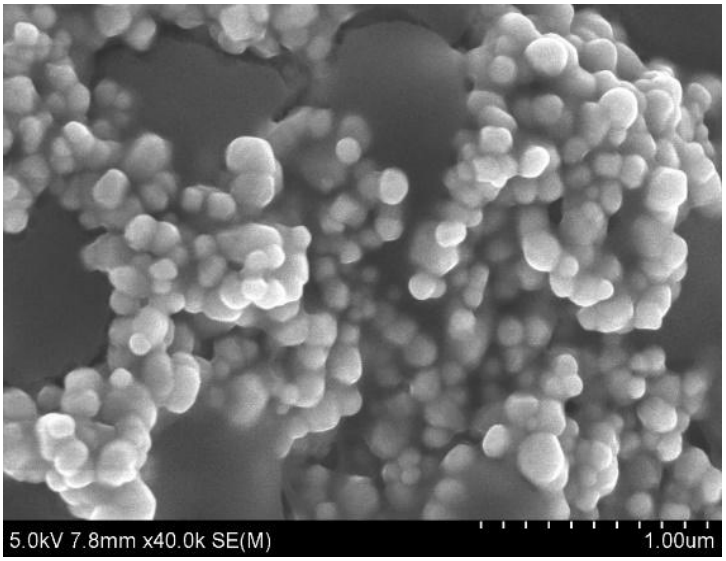
TiNPs powder



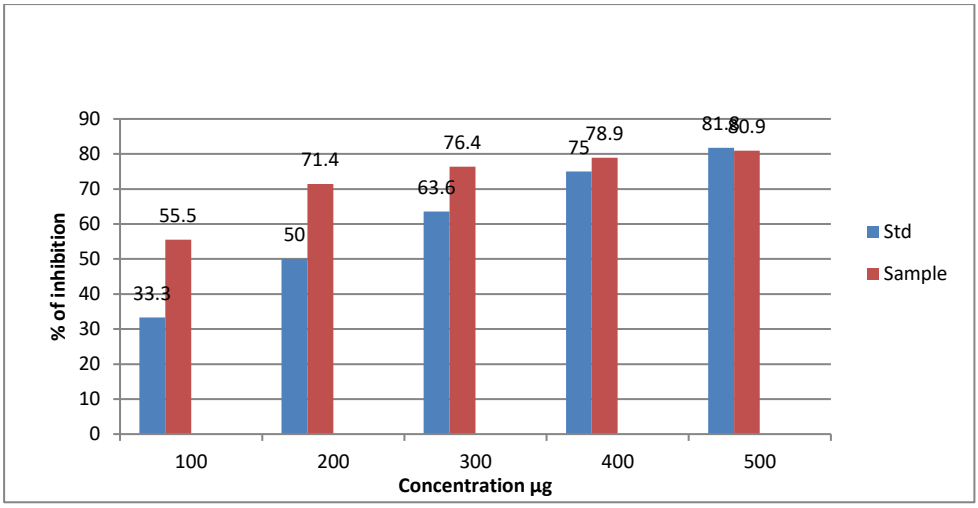
UV analysis of Titanium oxide nanoparticles



EDAX analysis of Titanium oxide nanoparticles



SEM analysis of Titanium oxide nanoparticles



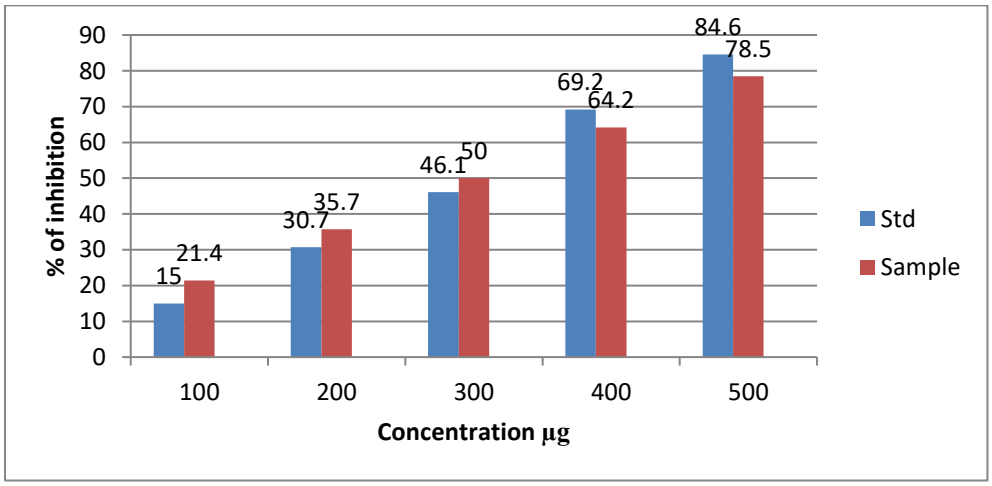
Alpha Amylase Assay

Table 1: Effect of Standard Compound on α-Amylase Activity at Different Concentrations

Concentration (µg)	Blank	100	200	300	400	500
Standard O.D	0.04	0.06	0.08	0.11	0.16	0.22
% of inhibition		33.3	50	63.6	75	81.8

Table 2: Inhibitory Activity of Test Sample on α-Amylase Enzyme at Various Concentrations

Concentration (µg)	Blank	100	200	300	400	500
Standard O.D	0.04	0.09	0.14	0.17	0.19	0.21
% of inhibition		55.5	71.4	76.4	78.9	80.9



Alpha Glucosidase Assay

Table 3: α-Glucosidase Inhibitory Activity of Standard Compound at Different Concentrations

Concentration (µg)	Blank	100	200	300	400	500
Standard O.D	0.13	0.11	0.09	0.07	0.04	0.02
% of inhibition		15	30.7	46.1	69.2	84.6

Table 4: In vitro α-Glucosidase Inhibition by Standard Compound at Increasing Concentrations

Concentration (µg)	Blank	100	200	300	400	500
Standard O.D	0.14	0.11	0.09	0.07	0.05	0.03
% of inhibition		21.4	35.7	50	64.2	78.5

Conclusion:

The pancreatic alpha-amylase, and alpha glucosidase break down the oligosaccharides and disaccharides into mono-saccharides suitable for absorption. The inhibition of these enzymes lowers the blood glucose level in blood. The alpha-amylase and alpha-glucosidase were inhibited in a concentration-dependent manner followed by various concentrations of TiONps. The enzymatic activity was lowered remarkably when the concentration of TiONps was increased. The IC50 value of TiONps synthesized from aqueous extract was higher both in alpha-amylase and alpha-glucosidase assays. The antidiabetic studies results proved that the Titanium oxide nanoparticles synthesized from aqueous extract of *Abutilon indicum* can be used to reduce blood glucose levels and inflammation. It has notable applications in the pharmaceutical and biomedical industries.

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MICROBIAL INFECTIONS OF THE NAIL: A REVIEW OF CURRENT PERSPECTIVES

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Abstract:

Microbial infections of the nail constitute a common yet challenging group of disorders affecting individuals across all age groups. These infections are primarily caused by fungi, bacteria, and viruses, with onychomycosis being the most prevalent condition worldwide. Nail infections not only result in cosmetic deformities but may also lead to pain, functional impairment, secondary infections, and a reduced quality of life, particularly in elderly, diabetic, and immunocompromised patients. Accurate diagnosis is often difficult due to overlapping clinical features and the unique anatomical structure of the nail, which limits drug penetration and therapeutic efficacy. Conventional treatment strategies include topical and systemic antifungal agents; however, their clinical success is frequently hindered by prolonged treatment duration, adverse effects, poor patient compliance, high recurrence rates, and emerging antimicrobial resistance. Recent advances in diagnostic techniques, pharmacotherapy, and novel drug delivery systems have improved treatment outcomes, yet several limitations persist. This review provides a comprehensive overview of the etiology, pathogenesis, clinical manifestations, diagnostic approaches, and current treatment options for microbial nail infections.

Keywords: Nail Infections, Onychomycosis, Green Nail Syndrome, Paronychia, Dermatophytes

Introduction:

Microbial nail infections account for a large percentage of dermatological consultations globally, making them a major clinical and public health concern. Fungi, bacteria, and viruses are the main causes of nail infections, with onychomycosis being the most common. While bacterial diseases like paronychia are typically linked to Staphylococcus aureus and Pseudomonas aeruginosa, fungal nail illnesses are often linked to dermatophytes, yeasts, and non-dermatophyte molds. Nail pathology is also influenced by viral infections, such as human papillomavirus-induced periungual warts. Because the clinical characteristics of these infections frequently overlap, it can be difficult to make an accurate diagnosis, which can result in therapy that is either delayed or ineffective.

The nail unit consists of the nail plate, the surrounding soft tissues, and their associated vascular and nerve networks, all anchored to the distal phalanx. The nail plate is a layered, keratinized structure positioned over the nail matrix (accounting for approximately 15–25%) and the nail bed with its distal onychodermal band (comprising about 75–85%), extending to the hyponychium at its free margin. The distal portion of the matrix, known as the lunula, appears as a pale, half-moon–shaped area visible in certain digits. The nail plate is bordered by the proximal and lateral nail folds. The cuticle, or eponychium, arises from the proximal nail fold and adheres to the surface of the nail plate, forming a protective seal. The nail unit features a rich and intricate vascular network that maintains sufficient blood circulation, while the periungual soft tissues and nail folds are well innervated to provide sensory function.[1]

The mature nail organ comprises the nail matrix, the nail bed, the nail plate, and the nail folds. Nail disorders can develop at any stage of life. Approximately 50% of these conditions are caused by infections, while around 15% result from inflammatory or metabolic diseases. A smaller proportion, about 5%, is associated with malignancies or pigmentary abnormalities. The differential diagnosis of nail disorders often presents significant challenges and remains a frequent source of clinical uncertainty. [2]

Types of Nail Infections

Onychomycosis

Onychomycosis, particularly the distal lateral subungual type (DLSO), represents the most common nail infection worldwide, accounting for nearly 90% of toenail cases. The condition is characterized by nail discoloration, thickening, and onycholysis, and in advanced stages, it may extend to the adjacent skin. The main etiological agents responsible for onychomycosis include dermatophytes, non-dermatophyte molds (NDMs), and yeasts. When dermatophytes alone cause the infection, it is referred to as *tinea unguium*. Among the dermatophytes, *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the predominant species, responsible for approximately 60–70% of infections. Other dermatophytes, such as *Epidermophyton floccosum*, are less commonly involved, while rare cases are attributed to additional species. The most frequently encountered NDMs associated with onychomycosis include *Scopulariopsis brevicaulis*, *Acremonium* spp., *Aspergillus* spp., *Fusarium* spp., and *Neoscytalidium* spp. Yeast-induced onychomycosis, primarily caused by *Candida* species, occurs more frequently in fingernails, especially among individuals whose hands are frequently exposed to moisture. [3]

Green Nail Syndrome

Green Nail Syndrome (GNS), also known as chloronychia, is the most common bacterial nail infection caused by *Pseudomonas aeruginosa*. Management of this condition is often challenging, as there are limited treatment guidelines supported by clinical trials. The infection typically occurs in individuals whose hands are frequently exposed to water, soaps, and

detergents, or subjected to repeated mechanical trauma—conditions that are especially common among elderly individuals. Clinically, the infection is characterized by a distinct green or black discoloration of the nail plate, which should prompt suspicion of *Pseudomonas* involvement. [4]

Paronychia

Paronychia is defined as an inflammatory condition of the nail fold, which may or may not be associated with abscess formation. It is generally classified into two types: acute and chronic. Acute paronychia usually affects a single digit and results from an infection, whereas chronic paronychia persists for six weeks or longer, often involving multiple nails and typically arising from repeated exposure to chemical irritants. Acute paronychia commonly develops following trauma to the nail plate, cuticle, or nail fold, which compromises the protective barrier and allows bacterial invasion, leading to infection and inflammation. *Staphylococcus aureus* and *Streptococcus* species are the predominant causative agents, although polymicrobial infections, including anaerobic bacteria, are also observed—particularly in individuals with diabetes mellitus, frequent exposure to oral flora, or in those who use injection drugs. [5]

Risk Factors of Nail Infection

Several factors can predispose individuals to nail infections, either by damaging the nail structure or by facilitating microbial entry. The major risk factors include:

- **Accidental trauma** – Injuries to the nail or surrounding tissue can disrupt the protective barrier, allowing pathogens to invade.
- **Artificial nails and frequent manicures** – The use of artificial nails and repeated manicure procedures can cause microtrauma and increase susceptibility to infection.
- **Manipulation of hangnails** – Pulling or cutting hangnails (small tears in the eponychium) can create entry points for bacteria or fungi.
- **Occupational exposure** – Jobs involving frequent contact with water or irritants, such as bartenders, housekeepers, dishwashers, and laundry workers, elevate the risk of nail infections.
- **Onychocryptosis (ingrown nails)** – The inward growth of the nail into the surrounding skin can lead to inflammation and secondary bacterial infection.
- **Onychophagia (nail biting)** – Habitual biting of nails introduces oral flora to the nail bed, increasing the likelihood of infection. [6]

Epidemiology of Nail Infection

The epidemiology of nail infections, is influenced by various factors including age, genetics, and underlying health or lifestyle conditions.

Age:

Age plays a significant role in the prevalence of onychomycosis within a population. Although the condition is more common in adults, a recent retrospective analysis involving 100 pediatric

patients aged from birth to 17 years revealed that approximately one in nine children exhibited some form of nail alteration. These finding highlights that, although less frequent, nail infections can also affect the pediatric population.

Genetics:

Genetic predisposition is another important factor influencing the occurrence of onychomycosis. Studies have suggested that distal subungual onychomycosis (DSO) caused by *Trichophyton rubrum* tends to show a familial pattern. In such cases, every child diagnosed with DSO had at least one parent with the same condition, indicating a possible hereditary susceptibility to fungal nail infections.

Smoking and Peripheral Arterial Disease:

Smoking and peripheral arterial disease (PAD) have been identified as independent predictors of onychomycosis. In a study involving 254 participants, abnormal nail changes and confirmed onychomycosis were observed in 49.2% and 22.4% of patients, respectively. Notably, 83.3% of patients who smoked two or more packets of cigarettes daily had onychomycosis, compared with only 14.8% among nonsmokers. Furthermore, extensive onychomycosis has been associated with immunodeficiency, as it occurs more frequently in individuals with acquired immunodeficiency syndrome (AIDS). [7]

Pathophysiology of Nail Infection

Entry of the Pathogen

Fungal invasion typically begins at the distal or lateral edge of the nail plate, where minor trauma, moisture, or disruption of the nail barrier facilitates access to the nail bed. Dermatophytes such as *Trichophyton rubrum* commonly penetrate through the stratum corneum of the nail bed or hyponychium. In immunocompromised individuals, the proximal nail fold may serve as the portal of entry, leading to proximal subungual onychomycosis

Adherence to Keratin

After penetration, fungi bind to hard keratin through surface adhesion molecules. Dermatophytes produce several keratinolytic enzymes including keratinases, proteases, lipases, and sulfatases that enable them to utilize nail keratin as a nutrient source. The nail plate, with its dense keratin structure and slow growth rate, provides an ideal environment for fungal persistence [8]

Nail Keratin Invasion

Once adherence is achieved, fungi migrate through the nail keratin layers. Three major patterns of invasion occur:

Distal–Lateral Subungual Onychomycosis (DLSO)

The most common form, where fungi spread under the nail plate from the distal or lateral edges. The infection extends proximally along the nail bed, leading to subungual hyperkeratosis and onycholysis [9].

Proximal Subungual Onychomycosis (PSO)

Fungi invade the nail unit through the proximal nail fold. This pattern is more common among individuals with compromised immunity [11].

White Superficial Onychomycosis (WSO)

Characterized by direct invasion of the superficial nail plate, producing white, chalky patches. The infection is largely restricted to the dorsal layers of the nail.

Immune Evasion Mechanisms

Dermatophytes employ several strategies to escape host immune responses:

- Mannan in their cell wall suppresses lymphocyte proliferation and reduces keratinocyte immune signaling.
- Production of biofilms, particularly by *Candida spp.* and some NDMs, enhances antifungal resistance and chronicity.
- Low vascularity of the nail apparatus limits immune cell access, promoting persistent infection [10].

These mechanisms enable the infection to remain minimally inflammatory yet long-lasting.

Tissue Damage and Nail Changes

As the fungi digest keratin and proliferate, they produce structural and functional nail changes:

- Thickening of the nail plate due to reactive hyperkeratosis
- Onycholysis resulting from separation of nail plate and bed
- Discoloration (yellow, brown, white, or black)
- Brittle, friable nail surface
- Accumulation of keratinous debris under the nail

These changes occur from both enzymatic degradation of keratin and mechanical disruption of nail architecture.

Chronicity and Recurrence

Onychomycosis is often chronic because nails grow slowly and fungal spores remain viable for extended periods. Reinfection can occur from contaminated footwear, communal bathing areas, or persistent environmental reservoirs. Inadequate perfusion in elderly and diabetic individuals further contributes to chronic infection and delayed recovery [9]

Etiology of nail infection

Onychomycosis (fungal nail infection) is caused by **dermatophytes, non-dermatophyte molds (NDM), and yeasts**.

- **Dermatophytes**: *Trichophyton rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum* are the most common.

- **Non-dermatophyte molds (NDM)**: *Aspergillus spp.*, *Scopulariopsis brevicaulis*, *Fusarium spp.*, *Acremonium spp.*, and *Neoscytalidium spp.*
- **Yeasts**: *Candida albicans*, *C. parapsilosis*, and *C. tropicalis*, more common in fingernails, especially among individuals frequently exposed to water.

Predisposing factors: diabetes mellitus, peripheral vascular disease, immunosuppression (HIV, corticosteroid therapy), trauma, occlusive footwear, and prolonged exposure to moist environments. [12][13]

Symptoms of nail infection

- Nail discoloration (white, yellow, brown, or black)
- Nail thickening or deformity
- Brittleness or crumbling of the nail plate
- Onycholysis (separation of nail from nail bed)
- Subungual hyperkeratosis (debris accumulation under the nail)
- Pain or discomfort, especially in advanced cases [14]

Testing and Diagnosis

Clinical Examination

- Visual inspection of nail plate and surrounding tissue.
- Assessment for nail discoloration, thickening, and subungual debris. (15)(16)

Laboratory Diagnosis

Test	Description	Advantage
KOH Mount test	Nail scrapings examined under microscope after KOH digestion.	Rapid, simple, inexpensive
Histopathology (PAS Stain)	Detects fungal hyphae in nail tissue.	High sensitivity
PCR-Based Methods	Detects fungal hyphae in nail tissue.	Fast accurate species identification

Current Conventional treatment

Current conventional treatment of nail infections, particularly onychomycosis, primarily involves topical and systemic antifungal therapies. Topical treatment options include ciclopirox olamine 8% nail lacquer, amorolfine 5% nail lacquer, efinaconazole 10% solution, and tavaborole 5% solution, which are commonly prescribed for mild to moderate infections. However, their clinical effectiveness is often limited by poor penetration through the dense nail plate, the need for prolonged application for 6–12 months, and a high rate of recurrence. Systemic or oral antifungal agents such as terbinafine, itraconazole, and fluconazole are preferred for severe or extensive nail involvement due to better efficacy. Nevertheless, systemic therapy is associated with

significant drawbacks, including hepatotoxicity, potential drug–drug interactions, and contraindications in patients with liver disease or during pregnancy. Additionally, relapse and emerging antifungal resistance remain major challenges, with reported recurrence rates ranging from 10% to 53%, often resulting from reinfection or incomplete eradication of the pathogen. The extended duration of therapy and high treatment costs further contribute to poor patient compliance, ultimately affecting therapeutic outcomes. [17, 18]

Conclusion:

Microbial nail infections remain difficult to manage due to the protective nail barrier, prolonged treatment duration, and high recurrence rates. Although topical and systemic therapies are widely used, their effectiveness is limited by poor nail penetration, adverse effects, and patient non-compliance. Systemic antifungals pose safety concerns, while topical agents require long-term application. Relapse and emerging resistance further complicate treatment outcomes. Hence, there is a need for safer, more effective, and patient-friendly therapeutic strategies with improved nail drug delivery.

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COMPUTER-AIDED DRUG DESIGN (CADD): PRINCIPLES, METHODS, AND APPLICATIONS

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Abstract:

Computer-Aided Drug Design (CADD) has become an indispensable component in modern pharmaceutical research, revolutionizing the way novel therapeutics are discovered and optimized. CADD encompasses a broad suite of computational techniques—including structure-based drug design (SBDD), ligand-based drug design (LBDD), molecular docking, virtual screening, Quantitative Structure-Activity Relationship (QSAR) modeling, and pharmacokinetic profiling—that enable scientists to predict how small molecules interact with biological targets. These computational strategies significantly reduce the cost and time associated with traditional drug discovery, improve success rates in lead identification and optimization, and allow early assessment of ADME–Tox properties. Recent advances integrating machine learning and artificial intelligence have further enhanced CADD efficiency and predictive accuracy. Despite challenges such as protein flexibility and scoring function limitations, the multidisciplinary nature of CADD continues to push the boundaries of rational drug design. CADD also plays a critical role in drug repositioning and development against global health threats, including cancer, viral infections, and neurological disorders. This chapter presents a comprehensive overview of CADD concepts, methodologies, case studies, current limitations, and future directions in the context of pharmaceutical science.

1. Introduction:

The traditional drug discovery process is notoriously time-consuming and expensive, often taking over a decade and billions of dollars before a new chemical entity reaches the market. To address these challenges, researchers increasingly rely on Computer-Aided Drug Design

(CADD)—a multidisciplinary field that integrates computational chemistry, structural biology, bioinformatics, and cheminformatics to accelerate the identification and optimization of drug candidates. By simulating molecular interactions in silico, CADD complements experimental approaches, enabling rational and efficient lead discovery with improved success rates in later phases of development.

2. Historical Background and Evolution

CADD has evolved significantly since its inception in the late 20th century, driven by advancements in computational power, structural biology, and database availability. Early methods focused on simple models of structure affinity relationships, whereas modern CADD incorporates sophisticated physics-based simulations, machine learning, and artificial intelligence to model complex biomolecular systems. The integration of diverse computational techniques allows researchers to more accurately predict drug-target interactions and optimize molecular properties.

3. Fundamental Concepts in CADD

3.1. Drug Discovery Workflow

CADD supports multiple stages of the drug discovery process:

- Target identification and validation
- Lead identification
- Lead optimization
- Preclinical assessment

At each stage, computational models accelerate decision-making and minimize experimental resource use by focusing on the most promising candidates.

3.2. Structure-Based Drug Design (SBDD)

SBDD leverages the three-dimensional structure of a biological target, usually determined by X-ray crystallography or NMR spectroscopy. Key techniques include:

- **Molecular docking:** Simulates how small molecules (ligands) bind within a target's active site.
- **Molecular dynamics (MD) simulations:** Explore the dynamic behavior of biomolecular complexes over time.
- **De novo design:** Generates new molecules based on structural features of targets.

These tools help predict binding affinity and orientation, enabling scientists to optimize interaction strength and specificity.

3.3. Ligand-Based Drug Design (LBDD)

When structural information of the target is unavailable, LBDD approaches analyze known active compounds to infer features associated with biological activity:

- **Quantitative Structure-Activity Relationship (QSAR):** Quantitatively correlates chemical features with activity.
- **Pharmacophore modeling:** Identifies spatial arrangements of molecular features essential for activity.
- **Similarity searching:** Selects compounds chemically similar to known actives.

These methods are particularly effective in exploring large compound libraries for candidate selection.

4. Key Computational Techniques

4.1. Molecular Docking

One of the most widely used CADD tools, molecular docking predicts how a ligand fits into the binding site of a target protein. Scoring functions evaluate the stability and affinity of the complex, guiding the selection of high-potential candidates. Docking is foundational in both SBDD and LBDD frameworks and is often paired with virtual screening.

4.2. Virtual Screening

Virtual screening enables the evaluation of millions of compounds against a target in silico, significantly reducing the experimental screening burden. It can be:

- Structure-based virtual screening (SBVS)
- Ligand-based virtual screening (LBVS)

Both approaches narrow down extensive chemical libraries to a manageable number of high-quality leads.

4.3. Pharmacokinetic and Toxicity Predictions (ADME–Tox)

Early assessment of absorption, distribution, metabolism, excretion, and toxicity properties (ADME–Tox) is essential to eliminate compounds likely to fail in later stages. Computational tools now enable in silico prediction of ADME profiles to filter out unsuitable candidates early.

4.4. Homology Modeling

When a target's 3D structure is unavailable, homology modeling builds structural models based on sequences of related proteins, enabling downstream SBDD techniques like docking.

4.5. Machine Learning and AI in CADD

Recent progress in machine learning (ML) has transformed CADD—improving predictions of binding affinity, generating novel molecular structures (de novo design), and optimizing leads via deep learning models. These tools increasingly supplement physics-based methods for enhanced accuracy and throughput.

5. Applications of CADD

5.1. Drug Repositioning

CADD facilitates drug repositioning—finding new therapeutic uses for existing drugs. Computational screening of known drugs against novel targets can rapidly identify potential

repurposing candidates with lower development risk and cost. For example, recent CADD studies targeted COVID-19 and cancer pathways.

5.2. Anticancer Drug Design

CADD has proven vital in designing inhibitors targeting oncogenic proteins such as RAS, improving drug specificity and reducing off-target effects. Molecular modeling and virtual screening refine inhibitor libraries tailored to specific cancer targets.

5.3. Antiviral and Antimicrobial Agents

The efficiency of CADD is demonstrated in infectious disease contexts where rapid identification of lead compounds is critical. By simulating interactions between viral proteins and potential drugs, CADD accelerates hit discovery and optimization.

5.4. Precision Medicine and Personalized Therapeutics

Incorporating genomic and proteomic data into computational models allows customization of drug design for individual genetic profiles, promoting personalized therapeutic strategies.

6. Advantages and Limitations

6.1. Advantages

- **Cost and time efficiency:** Computational predictions minimize experimental burden.
- **Targeted screening:** In silico prioritization focuses resources on promising leads.
- **Early elimination of non-viable candidates:** ADME-Tox profiling reduces late-stage failures.
- **Integration with experimental data:** Computational insights guide experimental design.

6.2. Limitations

Despite successes, CADD faces challenges:

- **Protein flexibility and dynamics:** Many models oversimplify dynamic interactions.
- **Scoring inaccuracies:** Docking scoring functions may not perfectly reflect real binding energies.
- **Data quality dependency:** ML models require high-quality datasets for reliability.

Ongoing research is focused on overcoming these hurdles through enhanced algorithms, better integration of physics-based methods, and richer training datasets.

7. Case Studies and Success Stories

7.1. HIV Protease Inhibitors

CADD contributed to the optimization of early HIV protease inhibitors, demonstrating that rational design can enhance efficacy and specificity.

7.2. Influenza and COVID-19 Therapeutics

Computational modeling helped identify candidate molecules targeting viral proteins in influenza and SARS-CoV-2, illustrating CADD's relevance to emerging health threats.

7.3. Lead Optimization Using AI

Advanced generative AI models are now being applied to refine lead molecules, demonstrating how deep learning facilitates structural modification for enhanced binding. (Example research: generative deep learning for structural modification).

8. Future Perspectives

CADD continues to evolve through:

- **Integration with AI/ML:** Enhancing predictive accuracy and automation.
- **Quantum computing applications:** Enabling faster and more precise simulations.
- **Better scoring functions and dynamic models:** Improving reliability of predictions.
- **Cloud and high-performance computing:** Expanding computational capacity for large-scale screenings.

These trends suggest a future where computational methods are deeply embedded in routine drug discovery workflows, further reducing the gap between discovery and clinical application.

Conclusion:

Computer-Aided Drug Design has transformed pharmaceutical research by introducing rational, efficient, and cost-effective methodologies for drug discovery and optimization. By integrating structural biology, cheminformatics, and advanced computation, CADD serves as an indispensable tool bridging theory and experiment. As computational power and algorithms improve, the impact of CADD will only grow, driving innovation across therapeutic domains and enabling more personalized, effective medicines.

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A SHORT NOTES ON OLDROYD'S AND WALTERS LIQUID MODEL

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Oldroyd's Liquid:

Oldroyd liquid is usually used to model and study the nature of visco-elastic properties. It also helps in explaining normal stress differences and stress relaxation. In industry oldroyd liquid is used to make paints, inks, adhesives, shampoos and cosmetics.

In 1906 and 1911, Einstein tried to explain the nature of colloids and suspensions containing large number of rigid spherical particles of same radii in a Newtonian fluid of viscosity η. He observed that the effective viscosity of these fluid mixtures is

$$\eta^* = \eta\left(1 + \frac{5}{2}a^3\right)$$

provided $a^3 \ll 1$

Frohlich and Sack (1946) explained the flow behavior of these mixtures by assuming the elastic spherical particles. They studied this new system by considering the constitutive equation

$$\tau' + \lambda_1\dot{\tau}' = 2\eta^*(e + \lambda_2\dot{e}) \quad (1)$$

where λ_1 and λ_2 are known as stress relaxation constants and strain rate relaxation constants. Maxwell in 1867 considered a combination of mechanical models for Newtonian fluid and Hookian solid to understand the nature of colloids and suspension. He also derived the constitutive equation for this mechanical model

$$\tau' + \lambda_1\dot{\tau}' = 2\eta e \quad (2)$$

This constitutive equation (2) explains the stress relaxation effect. In 1929, Jeffrey extended the constitutive equation (2) by considering the effects of strain-rate relaxation also and he proposed the new constitutive equation to study the behavior of high polymer solutions

$$\tau' + \lambda_1\dot{\tau}' = 2\eta(e + \lambda_2\dot{e})$$

These material constants $\eta, \lambda_1, \lambda_2$ are calculated experimentally by Toms and Strawbridge (1953) in the analysis of flow patterns of polymethyl methcrylate and n-butyl acetate.

Oldroyd in 1950 gave the suggestion of generalizing this one dimensional empirical rheological equations of state. He defined the convected differentiation of a mixed tensor $b_{i..}^{k..}$ as

$$\frac{\delta}{\delta t}b_{i..}^{k..} = \frac{\partial b_{i..}^{k..}}{\partial t} + v^m b_{i..,m}^{k...} + \sum v_{,i}^{m} b_{m..}^{k..} - \sum' v_{,m}^{k} b_{i..}^{m..}$$

where $\sum$ & $\sum'$ denote the sum of all similar terms, one for each covariant (contravarient) suffix.

Oldroyd used this convected differentiation to generalize the constitutive equation (1) in many ways. One possible generalization is

$$\left(1+\lambda_1\frac{\delta}{\delta t}\right)\tau'_{ik}=2\eta\left(1+\lambda_2\frac{\delta}{\delta t}\right)e_{ik}\ (3)$$

Another possible generalization is

$$\left(1+\lambda_1\frac{\delta}{\delta t}\right)\tau'^{ik}=2\eta\left(1+\lambda_2\frac{\delta}{\delta t}\right)e^{ik}\ (4)$$

These constitutive equations (3) and (4) represent different liquids but the one dimensional model for both of the two liquids is same. The liquids satisfied these constitutive equations are named as Oldroyd's liquid A and Oldroyd's liquid B. The constitutive equations for these fluids are linear.

Oldroyd again in 1958 gave a generalization of these two linear constitutive equations as

$$\tau_{ik}+\lambda_1\left[\frac{\delta}{\delta t}\tau_{ik}-\tau_{ij}e_{jk}-\tau_{jk}e_{ij}\right]+\mu_0\tau_{ij}e_{ik}+\nu_1\tau_{ji}e_{ji}\delta_{ik}$$

$$=2\eta_0\left[e_{ik}+\lambda_2\left\{\frac{\delta}{\delta t}e_{ik}-2e_{ij}e_{jk}+\nu_2e_{je}e_{je}\delta_{ik}\right\}\right]\ (5)$$

This equation (5) satisfied both Oldroyd's liquid A and Oldroyd's liquid B. It will govern Oldroyd liquid A, if

$$\eta_0>0,\lambda_1=-\mu_1>\lambda_2=-\mu_2\geq 0,\mu_0=\nu_1=\nu_2=0\ (6)$$

and liquid B, if

$$\eta_0>0,\lambda_1=\mu_1>\lambda_2=\mu_2\geq 0,\mu_0=\nu_1=\nu_2=0\ (7)$$

The constitutive equation (5) leads to the generalized Oldroyd's liquid. The equation (5) explained lots of important features of non-Newtonian fluid. A number of fluid flow problems have been solved by studying the effect of elastic as well as these non-linear terms present in the equation (5). Tanner explained Rayleigh problem (1962) and Helical flow (1963), Lesile (1961) studied the behavior of slow fluid flow past a sphere, Torsional oscillations of disk (1964_1) and sphere (1964_2) were analyzed by Frater, the fluid flow near a stagnation point was derived by Sharma (1959) and Nanda (1963) examined the fluid flow due to vibrating plane by using the generalized constitutive equation given by Oldroyd.

Walters liquid

Walters liquid also known as non-Newtonian visco-elastic liquid is used to describe fluid models that exhibit short term memory elastic effects. It is mainly used in dilute and semi dilute polymer solution, in paints, vanishes, coatings etc.

In 1960, Walters derived a constitutive equation by considering infinite number of Maxwell elements connected in parallel and the constitutive equation is

$$\tau'_{ik}(x,t) = 2\int_{-\infty}^{t} \psi\,(t-t')e_{ik}(x,t')dt' \quad (8)$$

$$\psi(t-t') = \int_{0}^{\infty} \frac{N(\lambda)}{\lambda} e^{-\frac{t-t'}{\lambda}} d\lambda \quad (9)$$

where $N(\tau)$ is the relaxation spectrum.

Walters further generalized the constitutive equation by replacing the ordinary integral (8) with Oldroyd's convected one and finally the two generalized constitutive equations are given as follows:

$$\tau'_{ik}(x,t) = 2\int_{-\infty}^{t} \psi\,(t-t')\frac{\partial x'^m}{\partial x^i}\frac{\partial x'^r}{\partial x^k} e_{mr}(x',t')dt' \quad (10)$$

$$\tau'^{ik}(x,t) = 2\int_{-\infty}^{t} \psi\,(t-t')\frac{\partial x^i}{\partial x'^m}\frac{\partial x^k}{\partial x'^r} e^{mr}(x',t')dt' \quad (11)$$

Walters referred the fluids governed these constitutive equations as liquids A′and B′respectively. Walters has studied a number of flow problems with these constitutive equations. The fluid flow through curved pipes of circular (1963) and elliptical (1965) cross sections was studied by Thomas and Walters by using the constitutive equation (11). Beard and Walters (1964) derived the two-dimensional boundary layer equations and using these equations discussed the flow near a stagnation point.

Walters in 1962, showed that for liquids with short memories, the equation of state can be simplified to

$$\tau^{ik} = 2\eta_0 e^{(1)ik} - 2k_0\frac{\delta}{\delta t} e^{(1)ik} \quad (12)$$

where $\eta_0 = \int_0^\infty N(\lambda)d\tau$ is the limiting viscosity at small rate of shear, $k_0 = \int_0^\infty \lambda N(\lambda)d\tau$

The convected differentiation of any contravarient tensor is given by

$$\frac{\delta b^{ik}}{\delta t} = \frac{\partial b^{ik}}{\partial t} + v^m\frac{\partial b^{ik}}{\partial x^m} - \frac{\partial v^k}{\partial x^m}b^{im} - \frac{\partial v^i}{\partial x^m}b^{mk} \quad (13)$$

where v_i is the velocity vector. This idealized model is a valid approximation of Walters liquid (Model B′) taking very short memories into account so that the terms involving

$$\int_{0}^{\infty} \lambda^n N(\lambda)\,d\lambda, n \geq 2$$

have been neglected.

The mixture of Polymethyl Methacrylate and pyridine at 25^0 C containing 30.5 gm of polymer per litre and having density 0.98 gm/ml. fits very nearly to this model. For this mixture, the relaxation spectrum as given by Walters is

$$N(\lambda) = \sigma\eta_0\delta(\lambda) + \frac{1-\sigma}{\beta}\eta_0 \ (0 \le \lambda \le \beta)$$

$$= 0 \ \tau < \beta$$

where $\sigma = 0.13, \eta_0 = 7.9$ poises (gm/cm. sec), $\beta = 0.18$ sec and $\delta(\lambda)$ is the Dirac's delta function so that k_0=0.60557 gm/cm.

Conclusion:

Oldroyd liquid generally applicable when effect of elasticity is more whereas Walters liquid is preferred when effects of elasticity is less and short lived. Both liquid models play an important role in describing and predicting the nature of visco-elastic fluids in engineering, biomedical and industrial applications.

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ORGANIC (GRAPHENE AND CARBON) QUANTUM DOTS: SYNTHESIS, PROPERTIES AND BIOMEDICAL APPLICATIONS

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Abstract:
Organic (O) dots is a term used to represent materials including graphene quantum dots and carbon quantum dots because they rely on the presence of other atoms (O, H, and N) for their photoluminescence or fluorescence properties. They generally have a small size (as low as 2.5 nm), and show good photostability under prolonged irradiation. The excitation and emission wavelengths of O-dots can be tailored according to their synthetic procedure. A wide range of strategies have been used to modify the surface of O-dots for passivation, improving their solubility and biocompatibility, and allowing the attachment of targeting moieties and therapeutic cargos. In this chapter covers the synthesis, physics, chemistry, and characterization of O-dots and their applications in bioimaging and gene delivery.

Keywords: Graphene Quantum Dot (GQD), Carbon-Based Quantum Dot (CBQD), Photoluminescence, Gene Delivery, Bioimaging.

1. Introduction:

Nanomaterials are often used for drug delivery and cancer imaging due to the fact that they allow the synergistic combination of diagnosis and therapy in a single nanoplatform (Schroeder *et al.,* 2011). Among the carbon nanomaterials, carbon-based quantum dots (CBQDs) including graphene quantum dots (GQDs) and carbon quantum dots (CQDs) show beneficial properties of low toxicity, environmentally friendly nature, simple and cost effective synthetic routes, and comparable optical properties to conventional semiconductor quantum dots and organic dyes (Lim *et al.,* 2015). In the last few years, CBQDs have been utilized in different biomedical applications, and many reports have discussed their synthesis, functionalization, combinations and applications (Nekoueian *et al.,* 2019). Although graphene quantum dots and carbon quantum dots have attracted significant attention, many researchers are still confused about the differences between these two sub-groups, and thus their correct and appropriate use in studies is challenging. GQDs are defined as graphene sheets with a size in the range of about 3–20 nm, which possess photoluminescence properties due to their physiochemical characteristics mostly because of their size, (Zhao *et al.,* 2020) while CQDs are photoluminescence spherical nanoparticles (Wang *et al.,* 2017). CQDs are also referred to as carbon dots in some studies.

However, there are some significant chemical, physical and optical differences between GQDs and CQDs, which will be discussed below. In this chapter, summarize the recent advancements in the design and applications of graphene and carbon quantum dots including bio-imaging, and gene delivery.

2. Chemical and Physical Properties of Organic Dots

GQDs, are composed of a single or multiple graphene layers with chemical groups attached to the edges, and they are commonly anisotropic, with lateral dimensions much larger than their thickness. GQDs are synthesized from pristine few-layer-thick graphene flakes as a precursor (Yan *et al.,* 2013). They have a narrow size distribution of 3–8 nm and small sheet shaped morphology, as shown by transmission electron microscopy (TEM) imaging. Due to the presence of a carbon core, GQDs have a crystalline structure with a lattice spacing of 0.24 nm comparable to the (100) facet of graphite and a honeycomb lattice with zigzag edges of 7 nm GQDs. This is in contrast to the spherical CQDs that have been formed using ribonuclease A as a bimolecular templating agent under microwave irradiation, which have an interlayer distance of 0.34 nm, matching the (002) facet of graphite (Yan *et al.,* 2013). The chemical composition of organic dots can be obtained from Fourier-transform infrared spectroscopy (FTIR). The three strong bands at around 3425, 1720 and 1645 cm^{-1} shown in the FTIR spectrum of the GQDs synthesized by an electrochemical method are associated with the vibrations of the hydroxyl (–OH), carbonyl (CQO) and graphitic (CQC) groups, respectively. The band at 1078 cm^{-1} is related to the alkoxy groups (O–C–O) present in the GQDs. The spectrum reveals that GQDs have many oxygenated functional groups on their surface. Their FTIR spectrum is also significantly different from that of the precursor graphene flakes, with a weak adsorption band at around 3425 cm^{-1}. The FTIR spectrum of CQDs depicts similar features of distinct strong bands at 3465 cm^{-1} (OH vibration) and 1618 cm^{-1} (CQO) with additional weak bands attributed to graphitic CQC (1645 cm^{-1}), O–C–O (1078 cm^{-1}) and B–O (1090 cm^{-1}), and stretching and deformation vibration modes of the boroxol bond of the boronic acid moieties (Wang *et al.,* 2016). The presence of C–N is demonstrated by the peak at 1400 cm^{-1}. Raman analysis of these organic quantum dots displays the carbon characteristic D and G bands at around 1350 and 1570 cm^{-1}, respectively.

3. Optical properties

O-dots normally show strong optical absorption in the UV region (260–320 nm) due to the p–p* transition of their C=Cbonds. The shoulder peak located at 270–390 nm is attributed to the n–p* transition of the C=O bonds (Wu *et al.,* 2014). The location of the peak in the spectrum rather than the intensity is more affected by the preparation route (Tan *et al.,* 2015). Under UV irradiation, different emission colors have been observed for GQDs, which is related to the synthetic routes employed. For example GQDs can emit bright UV (Hao *et al.,* 2012), blue (Tetsuka *et al.,* 2012), green (Ding *et al.,* 2015), yellow (Li *et al.,* 2011), red (Yang *et al.,* 2015),

and near infra-red (Ge *et al.,* 2014), emissions. CQDs have only a single excitation peak, which triggers the maximum emission, but GQDs regularly show two separate excitation peaks. The zigzag edges of GQDs are triplets like carbene, where they possess both s–p and p–p* transitions, which have an energy difference of <1.5 eV between the two peaks (Ye *et al.,* 2011). The different aspects of the properties of O-dots are illustrated in Figure 1.

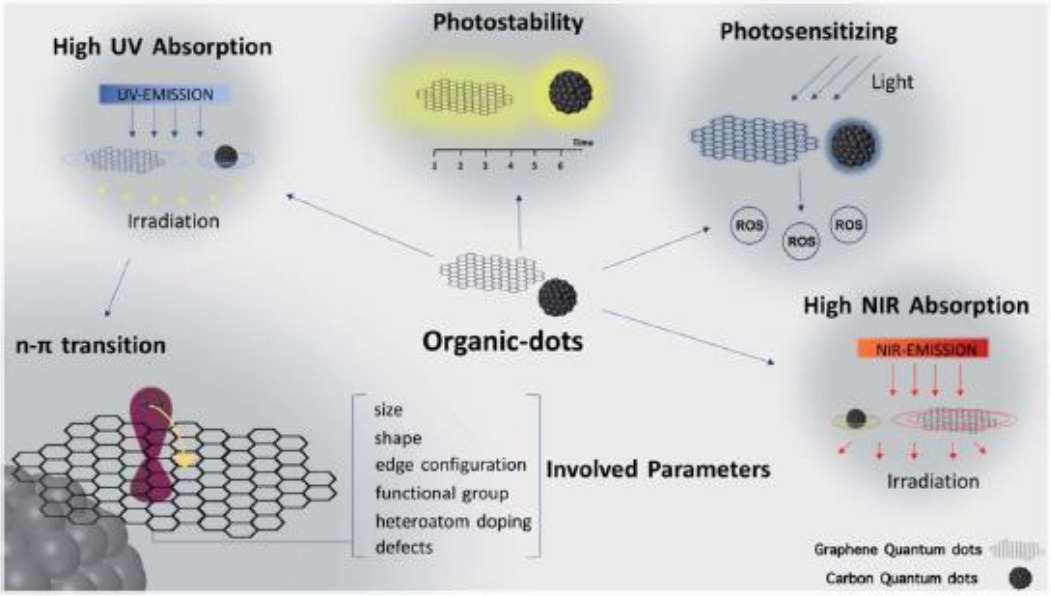

Figure 1: Properties of organic dots. Both GQDs and CQDs possess various optical properties including high UV and NIR absorption, photosensitizing nature and high photo-stability (Dezfuli *et al.*, 2021)

3. Effects of O-Dots on Cell Viability and Cytotoxicity

To investigate this toxicity, monitoring of body weight, blood chemistry panel, and hematology profile, and histological analysis are carried out (Nel *et al.,* 2012). For carbon-based quantum dots (Odots), both their in vitro and in vivo toxicity have been widely studied, which are highly dependent on their shape, size, and surface coating (Hong *et al.,* 2013) (Figure 2). Zboril and co-workers carried out an inclusive in vitro cytotoxicity study on mouse fibroblasts (NIH/3T3) using 3 types of CQDs, which were differed in their surface functionalization, providing overall negative, positive and neutral charges. The results suggested that the neutral carbon quantum dots had low toxicity and higher safety up to concentrations of 300 mg mL^{-1}.

However, the negatively charged carbon quantum dots caused morphological changes in the cells, and stimulated proliferation with higher levels of oxidative stress by interrupting the G2/M phase of the cell cycle; however, they did not enter the cell nucleus. In contrast, the positively charged CQDs showed the greatest toxicity to the cells due to the changes in the G0/G1 phase of the cell cycle, and they could also enter the cell nucleus, even at low concentrations (Hola *et al.,* 2016). In a study reported by (Wang *et al.,* 2018) the genotoxicity of GQDs towards NIH- 3 T3 cells was investigated by flow cytometry analysis for DNA damage-related protein activation,

while the GQD-induced ROS generation was studied as a potential explanation for DNA damage.

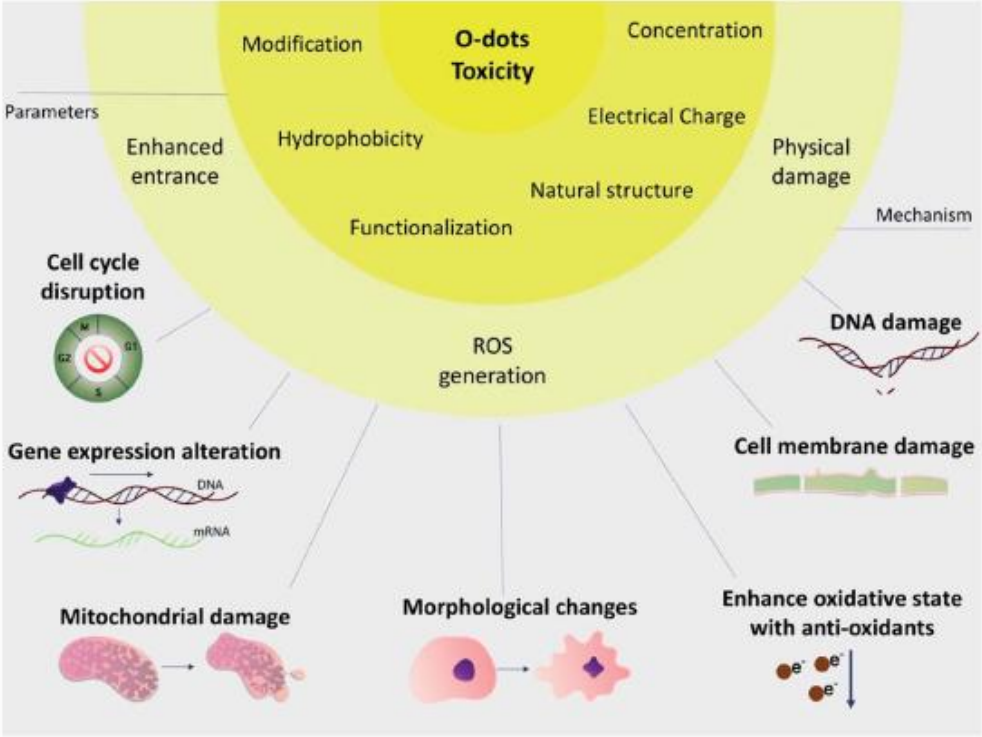

Figure 2: Toxicity of organic (O)- dots. The parameters involved in the toxicity of organic dots are demonstrated above. These parameters define the mechanism of impact and together cause damage and alterations in biological systems. Some of these effects lead to cell death, while others may cause malfunctioning (Dezfuli *et al.*, 2021)

The cellular uptake of GQDs and the cell death and proliferation of NIH-3T3 cells treated with GQDs were also studied to assess the cytotoxicity of GQDs (Chen *et al.,* 2015). The toxicity of GQDs to HeLa cells was tested using the CCK-8 assay, showing that the cell viability decreased with an increase in GQD concentration. More than 90% cell viability was observed at concentrations ranging from 21.5 to 50 mg mL^{-1}. Chong *et al.* reported a detailed and systematic study on the in vivo toxicity of GQDs (Ma *et al.,* 2014). In the case of O-dots, their electrical charge, functional groups, modification, morphology, hydrophobicity and concentration define the quality of the interaction between them and biological systems. Carbon based materials are active materials, and this property allows the possibility of their efficient functionalization and modification, but also allows them to take part in unwanted reactions with biological components. Thus, by manipulating these parameters, it will be possible to come up with a structure that possess a balance between maximum function and minimum toxicity.

4. Synthesis of Organic Quantum Dots

A variety of methods have been used for the preparation of O-dots (CQDs and GQDs) (Figure 3). In this section, we summarize the different approaches for the production of O-dots.

Regardless of the specific carbon nanostructure, the synthetic approaches can be categorized into two main categories, i.e. the top-down and bottom-up methods. Top-down methods are based on the progressive break down of larger carbon structures (e.g., graphite powder, carbon rods, carbon nanotubes, carbon black and even candle soot) (Liu *et al.,* 2007) by various methods including, laser ablation (Reyes *et al.,* 2016), hydrothermal (Hu *et al.,* 2013), electrochemical oxidation (Shinde *et al.,* 2012) and arc discharge (Dey *et al.,* 2014). This method offers some advantages such as abundant raw materials for the fabrication of O-dots, large-scale production, and simple operation. The obtained O-dots possess a highly crystalline nature and good aqueous dispersibility.

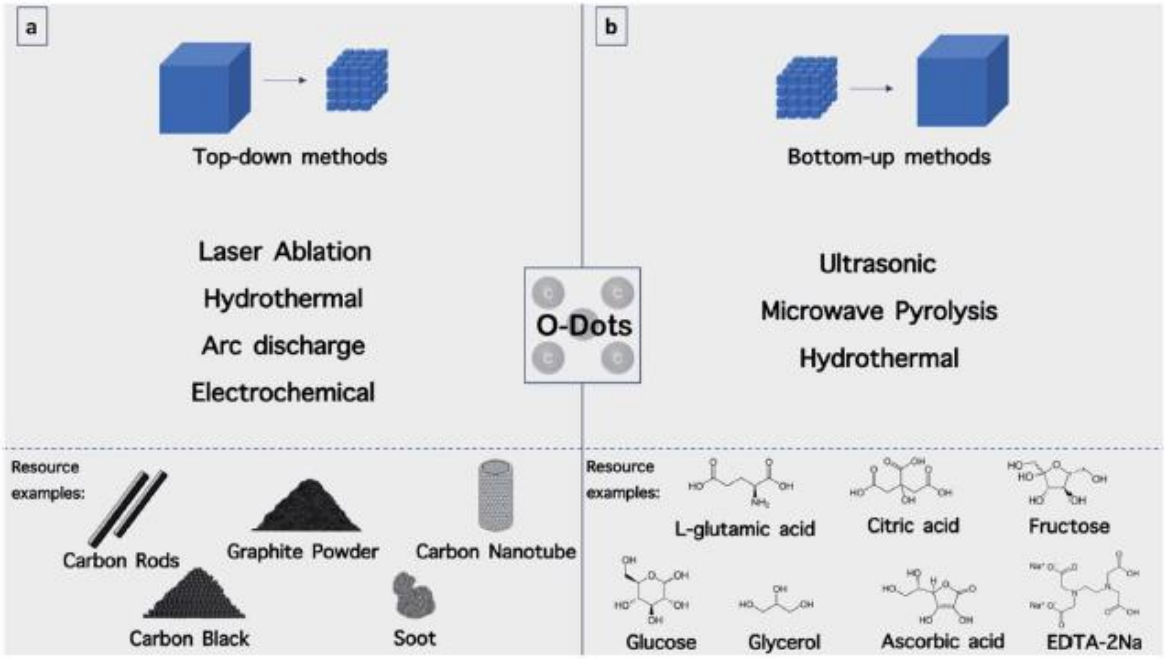

Figure 3: Synthetic approaches for O-dots. (a) Top-down methods including laser ablation, hydrothermal, arc discharge and electrochemical methods with mentioned resources. (b) Bottom-top methods including ultrasonic, microwave pyrolysis and hydrothermal methods with the mentioned carbon precursors (Dezfuli *et al.,* 2021)

However, these nanomaterials commonly demonstrate a predominant sp^2 hybridized carbon structure, and therefore often lack an efficient electron band gap to provide fluorescence. Therefore, the size and surface chemistry should be modified via oxidizing agents such as concentrated acids (HNO_3 and H_2SO_4/HNO_3 mixture). In this process, the bulk carbon materials are reduced into smaller fragments, while the surface is altered with oxygencontaining groups. This two-step route has become well established for the formation of GQDs. In the first step, graphite is converted into graphene oxide (GO) sheets using the Hummers' method, while the second step involves cutting the GO into GQDs using various methods (Nekoueian *et al.,* 2019). The bottom-up approach employs certain molecular precursors that can form O-dots after dehydration and carbonization procedures. In general, the best precursors possess –OH, –COOH,

–CQO, and NH_2 groups, which can be dehydrated at higher temperatures. There are numerous approaches to perform the dehydration and carbonization processes, including hydrothermal microwave-hydrothermal (Tang *et al.,* 2012), plasma-hydrothermal approaches (Wang *et al.,* 2012) (Figure 3b). These methods offer exciting opportunities to control the molecular size, and shape and fine-tune the physicochemical properties of O-dots.

5. Surface Engineering of O-dots

For better interacting with biological systems, modification of O-dots with different molecules and structures including biomolecules is necessary. Moreover, surface modification can alter the surface characteristics of a material to make it more suitable for a particular application (Qian *et al.,* 2013). For this purpose, the addition of functional groups on O-dots will facilitate this process. Also, functionalization may change some of their physical and chemical characteristics (Feng *et al.,* 2017). The surface functional groups available on the surface of O-dots depend on the type of precursors and the reaction conditions. The functional groups present on the surface of O-dots include –OH and –COOH depending on the degree of oxidation. These groups readily form hydrogen bonds with water molecules, and their presence leads to reasonable solubility in water. Furthermore, these groups play a vital role in the enhancement of the PL efficiency. Therefore, alterations in the degree of oxidation can affect the optical properties of the O-dots. The QY of GQDs increases with a reduction in the oxygenation rate of GQDs, while the emission wavelength will be shifted towards longer wavelengths (red-shift) by their oxidation (Zhu *et al.,* 2012). In addition to these beneficial functional groups, additional functionalization with other materials such as polyethylene glycol (PEG) is necessary to improve the biocompatibility and also the QY of organic dots. The need for surface passivation offers some constraints in the synthetic procedure, which increases the overall particle size, resulting in a deleterious effect on the applications in of O-dots in different fields. PEG molecules, which are applied as surface passivation agents, can increase the inherent fluorescence emission (Zheng *et al.,* 2015). In addition to PEG, other small molecules such as ethylene diamine, octadecylamine, and 2-(2-aminoethoxy)-ethanol have been covalently linked to the surface of O-dots via an amide bond. Surface passivation leads to hydrophilicity and hydrophobicity in organic dots based on the nature of the functional groups (Chen *et al.,* 2009). Furthermore, the addition follows. of heteroatoms (especially nitrogen) can improve the PL and QY of carbon dots. N-doping of GQDs increases their QY and produces a blue-shifted emission due to the strong electronwithdrawing ability of the N atoms within the conjugated C plane Hu *et al.,* 2013). To add catalytic functions to organic dots or to improve their PL properties, other elements have also been doped into CQDs and GQDs. S and N co-doped CQDs and GQDs can have a QY value as high as 73% and 71%, respectively. Thus far, different functional groups such as amine, carboxyl, quaternary ammonium and alkoxysilane have been coated onto the surface of O-dots

(Figure 4). The most common groups detected on the O-dot surface are amine and carboxyl, which allow the conjugation of organic, polymeric, inorganic or biological moieties (Du *et al.,* 2013).

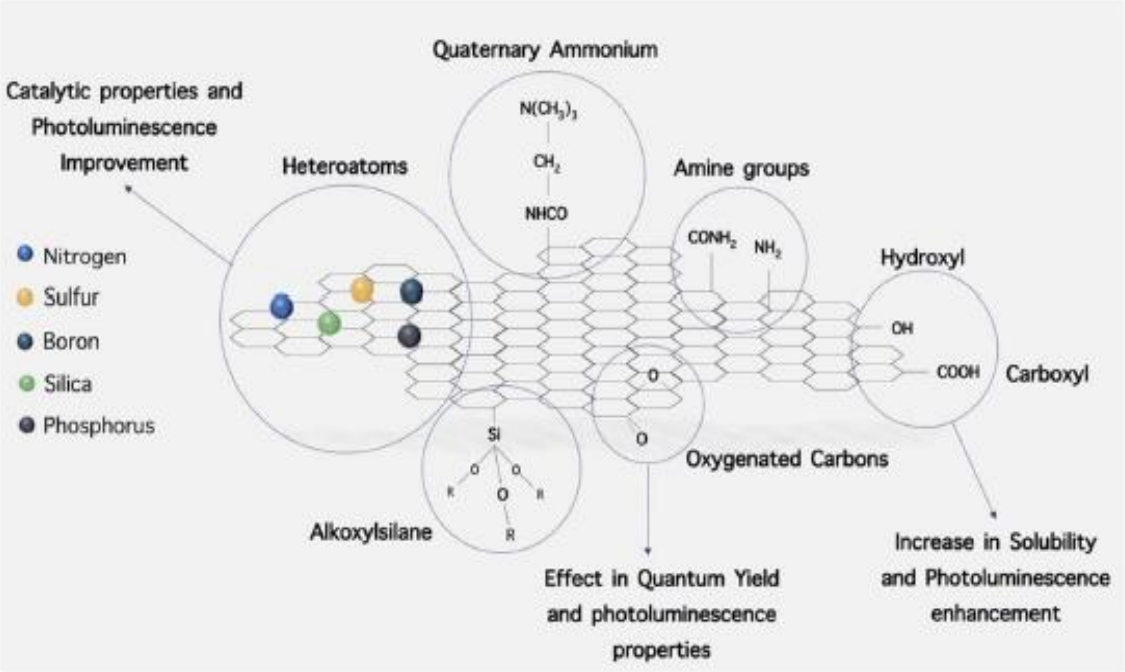

Figure 4: GQD functionalization and modification with other elements. Functional groups and heteroatoms add different properties to GQDs (i.e. increase in solubility, better photoluminescence and catalytic properties) (Dezfuli *et al.*, 2021).

6. O-dots and Biological Materials

The reactive groups present in the surface coating of O-dots can be modified according to the requirements. Numerous organic, polymeric, inorganic biological materials with specific functions can be attached on the surface of O-dots. Moieties can be attached to O-dots via several different interactions, including covalent bonds, electrostatic interactions and hydrogen bonds for specific applications.

7. Interactions of O-dots with Biomolecules

7.1. O-dot interaction with protein fibrils: The formation of peptide or protein fibrils within the extracellular space of tissue (Figure 5a) is believed to play a signficant role in the development of serious diseases such as Alzheimer's disease, Parkinson's disease and type-II diabetes. The formed mature protein fibrils are known to be cytotoxic and can provoke the death of affected cells (Glabe *et al.,* 2006). Therefore one preventative or therapeutic strategy for diseases associated with peptide or protein fibrillation is to inhibit or delay the fibrillation process. The possible application of O-dots for the inhibition of protein fibrillation was recently reported. Specifically, one new type of CQDs was prepared by (Li *et al.,* 2015) who investigated their effect on the fibrillation of human insulin. In this study, the formation of insulin fibrils was

significantly delayed when CQDs were added in a dose-dependent manner. No insulin fibrils were observed afteer incubation at 65 ºC for 5 days (i.e. 120 h) when a large concentration of CQDs (40 mg mL^{-1}) was present. Moreover, when the CQDs were covalently attached to other proteins (such as transferrin, human serum albumin, chicken ovalbumin, and hemoglobin), no protein fibrillation or conformational alteration was detected, even after 48 h at ambient temperature. Fluorinated graphene quantum dots (F-GQDs) are a new type of carbon nanomaterials with unique physicochemical properties due to their highly electronegative fluorine atoms. (Yousaf *et al.,* 2017) prepared highly fluorescent and water-dispersible F-GQDs using a microwave-assisted hydrothermal method, and investigated their inhibitory effect on the aggregation and cytotoxicity of hIAPP *in vitro*. The efficient inhibition of amyloid aggregation by the addition of F-GQDs was confirmed. In the presence of F-GQDs, the morphology of the hIAPP aggregates changed from entangled long fibrils into short thin fibrils and amorphous aggregates. By employing fluorescence analysis using thioflavin T, inhibited aggregation with a prolonged lag time, and reduced fluorescence intensity at equilibrium were demonstrated after hIAPP was incubated with the F-GQDs. Based on the circular dichroism spectrum results, the F-GQDs could inhibit the conformational transition of the peptide from its native structure to b-sheets. F-GQDs could also rescue the cytotoxicity of INS-1 cells induced by hIAPP in a dosedependent manner.

7.2. O-dot interaction with lipids: Another topic of interest is the possible interaction between O-dots and lipids or bio-membranes (Figure 5b). Recently, it was shown that CQDs could bind to the lipid bilayer and enter through cell-sized giantunilamellar vesicles. (Jelinek *et al.,* 2014) made significant contributions to elucidate the interaction of CQDs with lipids/membranes. In one study, they showed that amphiphilic CQDs could insert into the lipid bilayer of giant vesicles and induce FRET energy transfer from the CQDs to dyes embedded in the membrane. By conjugating the amphiphilic CQDs with phospholipids, the same group developed a new probe for the study of the environment and processes occurring in membranes (Mishra *et al.,* 2017). The interactions between the amphiphilic CQDs with actual bacterial cell membranes (not models) were also studied, in which the emission spectra of CQDs depended on the identity of the bacterial strains with regard to the spectral shift and peak intensity. Similar to CQDs, graphene quantum dots can also alter the structure of cell membranes. Jiang and co-workers assessed the toxicity of graphene oxide (GO) and nitrogen-doped graphene quantum dots (N-GQDs) on red blood cells (RBCs) through analysis of hemolytic activity. The morphology of the RBCs changed and their ATP content was lower after being exposed to the different nanomaterials. The structural changes of the RBC lipid membranes were studied via surface-enhanced infrared absorption spectroscopy using model membranes.

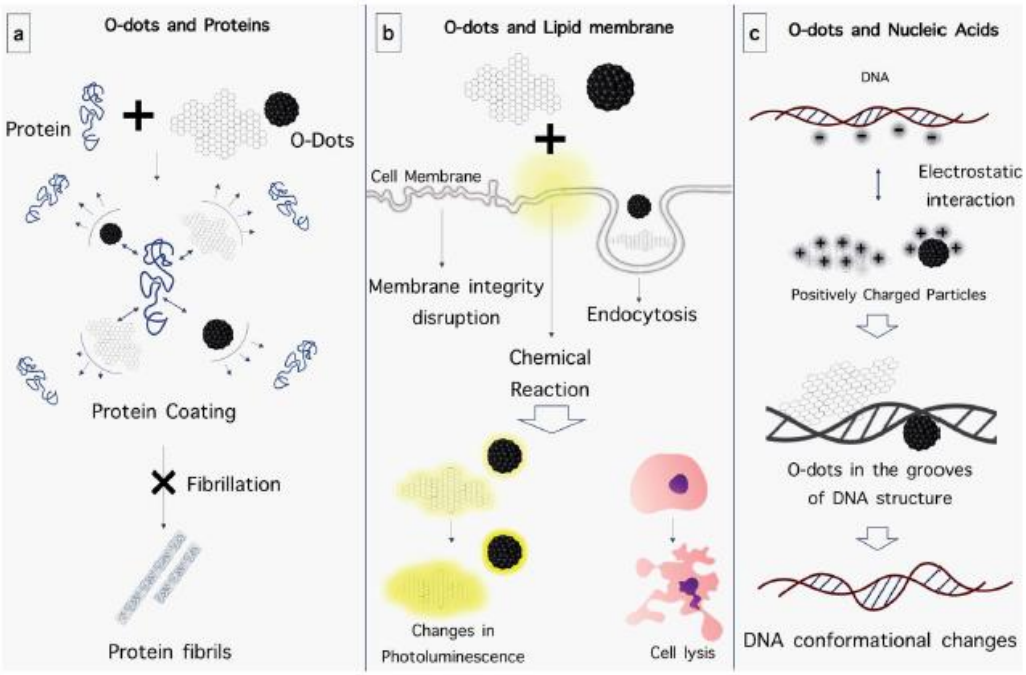

Figure 5: Interaction of O-dots with biological components. (a) O-dots prevent protein fibrillation by preventing the interaction of protein subunits with each other. (b) O-dots cause disruption in cellular membrane integrity, enter cells via endocytosis and cause chemical variations in membranes, leading to cell lysis and changes in photoluminescence. (c) O-dots attach to nucleic acids via electrostatic interaction and make conformational changes in them. (Dezfuli *et al.*, 2021)

7.3. O-dots interaction with nucleic acids. It is important to examine the interaction between nucleic acids (DNA and RNA) and different nanomaterials because nucleic acids areresponsible for carrying genetic information (Figure 5c). Recently, the interaction of CQDs with different types of DNA structures, i.e. double-stranded DNA (dsDNA) and singlestranded DNA (ssDNA) has attracted interest. Studies have shown that two different interaction mechanisms with CQDs can occur, producing discrete changes in fluorescence behavior. As an example, (Loo *et al.* 2016) prepared a sensing platform in which CQDs and a fluorescently labeled ssDNA probe (FAMLprobe) were used to identify a specific sequence of DNA. Due to the difference in the interactions of CQDs with ssDNA and dsDNA, the fluorescence of the FAMLprobe (ssDNA) was effectively quenched by CQDs, unless it had formed dsDNA by base-pairing with the target DNA. A recent study on the interaction of GQDs with three different types of DNA (well-matched DNA (WM-DNA), baseimpaired DNA (AB-DNA) and amino-modified DNA (AM-DNA)) showed that GQD could reduce the melting temperature of DNA, and thus reduce its stability. It was also observed that the WM-DNA structure and conformation were not changed, but AB-DNA changed after incubation with GQD.

8. O-dots as a Delivery Vehicle

The distinctive optical features of O-dots make them highly attractive candidates as imaging reporters combined with drug delivery and therapeutic applications. Their special properties allow them to overcome various problems associated with conventional imaging probes and to provide versatile nanoplatforms with both imaging and therapeutic capabilities.

8.1. Gene therapy

Gene therapy is considered a promising treatment for many diseases, but some barriers limit its clinical translation. Gene therapy involves the delivery of nucleic acid-based therapeutic agents into cells to modify the expression levels of various genes. Depending on the type of agent they can be activated in the cell nucleus or the cytoplasm. Genes, their expression, translation and transcription are a complicated system and their manipulations may cause serious implications on the functions of biological systems. Moreover, transferring a desired gene to the target properly without any damage to the gene is another limitation. Delivering other nucleic acids will also affect gene regulations in a complex manner. Therefore, gene delivery systems should be multifunctional besides being specific for certain targets and protective for their contents. Accordingly, GQDs and CQDs present a suitable function for this purpose due to their physiochemical properties. Gene delivery systems based on GQDs and CQDs should interact properly with nucleic acids, precisely deliver them to targeted cells, protect the nucleic acids from environmental conditions, not disrupt gene regulatory systems or other physiological process and cause proper gene expression or regulation. The parameters involved in efficient gene delivery are discussed below.

Ghafari *et al.* (2017) prepared peptide-modified GQDs with two different (green and red) emission colors, and tested them for gene delivery and nuclear targeting applications and cellular tracking. The GQDs were physically linked to the MPG-2H1 chimeric peptide, which contained three different amino acid sequence motifs. These motifs were designed to carry out three different functions, including, DNA packaging, endosomal escape, and nuclear targeting. These modified O-dots facilitated cell tracking and showed improved transfection of the luciferase plasmid into HEK 293 embryonic kidney cells.

Carbon quantum dots can also be applied as gene delivery platforms. Kim *et al.* (2017)292 reported the use of CQDs as a functional siRNA delivery system for effective gene knockdown in vitro and in vivo. They showed that CQDs increased the uptake of siRNA by tumor cells via endocytosis, accompanied by low cytotoxicity and interesting effects on the immune system. Fluorescence images verified the localization of the CQDs in the cytoplasm and release of siRNA within 12 h. The functional siRNA delivery system mediated by CQDs-PEI was used in an in vivo mouse model, with good gene knockdown efficacy and protection of the siRNA from degradation in vivo. Recently, (Yang *et al.* 2017) reported a simple one-step hydrothermal

carbonization procedure for the synthesis of positively charged CQDs using PEI and FA as carbon sources. The cytotoxicity experiments confirmed that the toxic effects of PEI were lower in the presence of CQDs, and the composite could be useful as a probe for the selective imaging of folate receptor (FR)-positive cancer cells compared to normal cells. The surface of CQD/pDNA had a positive charge, which facilitated the interaction with the weak negative charges of the cell membrane, suggesting that the surface of CQDs could efficiently capture pDNA molecules. To study gene expression in vitro, they used transfection with CQDs, and pDNA encoding enhanced green fluorescent protein (EGFP) in 293T and HeLa cells. These results showed that the positively-charged CQDs could efficiently transfect cells and could be useful for gene therapy.

8.2. Bioimaging applications

Other major application of GQDs and CQDs is bio-imaging. This application is based on their photoluminescence properties. (Dong *et al.,* 2012) incubated MCF-7 human breast cancer cells with GQDs synthesized via acidic oxidation, and a bright green emission could be detected using confocal laser scanning microscopy with excitation at 488 nm. The section analysis of single MCF-7 cells showed that GQDs not only labeled the cell membrane and the cytoplasm, but also the nucleus. This was the first time that luminescent carbon nanomaterials were shown to tag the cell nucleus. Moreover, Hela cells, A-549 cells, macrophages and hepatocellular cells have all been labeled with fluorescent GQDs by different groups. Peng *et al.* incubated green luminescent GQDs with the T47D human breast cancer cell line and stained their nucleus with DAPI. The images included a phase contrast, where the nuclei were stained blue with DAPI, and high contrast green fluorescent GQDs were observed in the perinuclear region in the triple over-layered images.

Conclusions:

In this chapter, summirized the recent progress in the design, properties, and theranostic applications of O-dots including graphene quantum dots and carbon quantum dots and introduce this new concept in order to facilitate new possibilities in the synthesis, preparation and application of these materials. These small-sized quantum dots have attracted much attention in theranostics due to their exceptional optical and chemical properties such as non-blinking, lowtoxicity and photostability. They are now being investigated in medical diagnostics and imaging, bio-sensing, drug/gene delivery, photoactivation, and light-activated therapy. Furthermore, O-dots can act as carriers for many pharmaceutical applications. Once functionalized with various polymers, they can serve as versatile biocompatible gene delivery vehicles. Moreover, they can be designed to have extended therapeutic lifetimes within the body. Despite some safety concerns about O-dots, many studies have reported their use in hybrid forms for biological applications, particularly in gene delivery. Hybridization approaches using

biocompatible polymers have been tested in order to lower their potential toxicity and to increase their biocompatibility and applicability as multi-functional imaging probes and delivery vehicles. However, more systematic toxicology studies are needed to confirm the safety and understand the pharmacokinetics of O-dots.

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OBESITY-INDUCED FATTY LIVER DISEASE

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Abstract:
Obesity has emerged as a major global health challenge of non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disorder worldwide. Obesity-induced NAFLD is now recognised as a complex, multisystem disease arising from metabolic dysregulation rather than simple hepatic fat accumulation. Excess adiposity, particularly visceral obesity, promotes insulin resistance, enhanced adipose tissue lipolysis, and increased flux of free fatty acids to the liver, leading to hepatic steatosis. Concurrently, adipose tissue dysfunction results in an imbalance of adipokines and the release of pro-inflammatory cytokines, fostering chronic low-grade inflammation, oxidative stress, and lipotoxicity within hepatocytes. These processes contribute to mitochondrial dysfunction, endoplasmic reticulum stress, and activation of inflammatory and fibrogenic pathways, driving disease progression from simple steatosis to non-alcoholic steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Emerging evidence also highlights the role of gut microbiota dysbiosis and the gut–liver axis in amplifying hepatic injury in obesity-associated NAFLD. It is strongly associated with type 2 diabetes mellitus, cardiovascular disease, and metabolic syndrome; obesity-induced NAFLD represents a significant clinical and public health burden. Understanding the underlying pathophysiological mechanisms is essential for the development of effective preventive and therapeutic strategies, with lifestyle modification and metabolic risk factor control remaining the cornerstone of current management.

Keywords: Obesity, Nonalcoholic Fatty Liver Disease, Insulin Resistance, Inflammation, Gut–Liver Axis, Metabolic Syndrome.

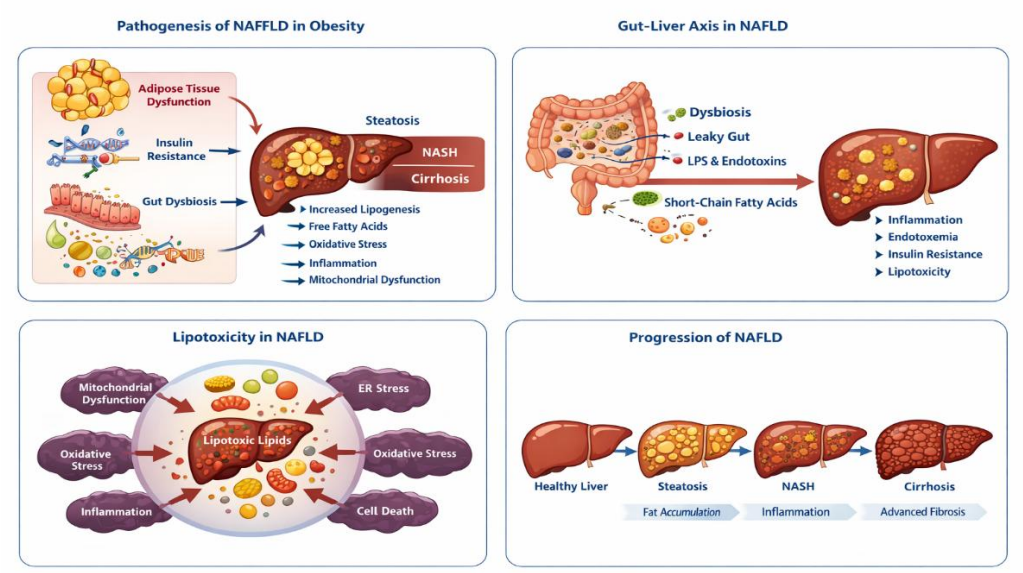
Introduction:

Obesity is no longer regarded as a simple disorder of excessive fat accumulation but is increasingly recognized as a chronic, relapsing metabolic and inflammatory disease. Among its numerous complications, obesity-induced fatty liver disease represents one of the earliest and most prevalent manifestations of metabolic dysfunction. The liver, as a central hub of lipid and glucose metabolism, becomes particularly vulnerable to the metabolic disturbances associated with obesity.

Non-alcoholic fatty liver disease (NAFLD), recently redefined under the broader terminology of metabolic-associated fatty liver disease (MAFLD), reflects a paradigm shift from exclusion-

based diagnosis to a positive metabolic framework. This redefinition highlights the strong causal relationship between obesity, insulin resistance, and hepatic steatosis. Importantly, obesity-induced fatty liver disease is now recognized not merely as a hepatic disorder but as a multisystem disease with implications extending to cardiovascular, renal, and endocrine health.

Despite advances in understanding its clinical spectrum, the molecular mechanisms driving disease progression remain incompletely elucidated.



2. Epidemiology and Global Health Impact

The prevalence of obesity-induced fatty liver disease parallels the global rise in obesity and metabolic syndrome. Epidemiological studies suggest that NAFLD affects nearly one-third of the adult population worldwide, with substantially higher prevalence among individuals with obesity, type 2 diabetes mellitus, and polycystic ovarian syndrome.

Notably, obesity-induced fatty liver disease is now a leading indication for liver transplantation in several regions and is projected to surpass viral hepatitis as the primary cause of end-stage liver disease. Beyond liver-related morbidity, NAFLD significantly increases the risk of cardiovascular disease, which remains the leading cause of mortality in this population. These findings emphasise the urgent need for early identification and targeted intervention strategies.

3. Obesity as a Metabolic Stressor

Traditionally, obesity has been attributed to excess caloric intake and reduced energy expenditure. However, contemporary evidence suggests that adipose tissue quality, rather than quantity alone, plays a crucial role in disease pathogenesis. In obesity, adipose tissue undergoes pathological remodeling characterized by hypoxia, fibrosis, immune cell infiltration, and altered adipokine secretion.

Visceral adiposity is particularly pathogenic due to its direct drainage into the portal circulation, exposing the liver to high concentrations of free fatty acids, inflammatory mediators, and gut-

derived endotoxins. This continuous metabolic stress primes the liver for steatosis and subsequent inflammatory injury.

4. Molecular Pathogenesis: A Multi-Hit and Systems Biology Approach

4.1 Dysregulated Lipid Flux and Metabolic Inflexibility

In obesity, the liver exhibits metabolic inflexibility, characterised by an impaired ability to switch between lipid and glucose utilisation. Excessive delivery of free fatty acids from adipose tissue, combined with increased hepatic de novo lipogenesis driven by hyperinsulinemia, overwhelms the liver's capacity to safely store or export lipids.

4.2 Lipotoxicity Versus Steatosis

While triglyceride accumulation was initially considered toxic, emerging evidence suggests that triglyceride storage may represent a protective mechanism. Instead, lipotoxic intermediates such as diacylglycerols, ceramides, and free cholesterol are now recognised as key mediators of hepatocellular injury, insulin resistance, and inflammation.

4.3 Mitochondrial Dysfunction and Energetic Failure

Obesity-induced fatty liver disease is associated with profound mitochondrial abnormalities, including reduced oxidative capacity, impaired mitochondrial biogenesis, and altered dynamics (fusion and fission). These defects lead to inefficient fatty acid oxidation and excessive production of reactive oxygen species, exacerbating cellular damage.

5. Immunometabolism and Chronic Low-Grade Inflammation

Obesity induces a state of chronic low-grade inflammation, often referred to as "metaflammation." In the liver, metabolic stress signals activate innate immune pathways, leading to the recruitment of macrophages, neutrophils, and lymphocytes. Kupffer cells shift toward a pro-inflammatory phenotype, amplifying cytokine production and sustaining tissue injury.

Recent studies highlight the role of inflammasomes, particularly the NLRP3 inflammasome, in linking metabolic stress to inflammatory signalling in fatty liver disease. Activation of these pathways contributes to hepatocyte ballooning, fibrosis, and disease progression.

6. Gut Microbiota and Metabolic Endotoxemia

Alterations in gut microbiota composition (dysbiosis) have emerged as a critical contributor to obesity-induced fatty liver disease. Dysbiosis leads to increased intestinal permeability, facilitating the translocation of bacterial products such as lipopolysaccharides into portal circulation. This phenomenon, termed metabolic endotoxemia, triggers hepatic inflammation via Toll-like receptor signalling.

Additionally, microbial metabolites such as short-chain fatty acids, bile acid derivatives, and ethanol produced by gut bacteria can modulate hepatic metabolism and inflammation. Targeting the gut–liver axis has therefore emerged as a promising therapeutic strategy.

7. Fibrosis Progression:

Fibrosis represents a maladaptive wound-healing response to chronic liver injury. In obesity-induced fatty liver disease, repeated cycles of hepatocyte injury and inflammation lead to sustained activation of hepatic stellate cells. These cells transform into myofibroblast-like cells, producing excessive extracellular matrix components.

Importantly, fibrosis stage—not steatosis or inflammation—is the strongest predictor of liver-related and overall mortality in patients with fatty liver disease, underscoring its clinical significance.

8. Emerging Biomarkers and Precision Medicine

Recent advances have identified novel non-invasive biomarkers for disease assessment, including circulating microRNAs, cytokeratin-18 fragments, metabolomic profiles, and imaging-based elastography techniques. These tools may facilitate early diagnosis, risk stratification, and monitoring of therapeutic response.

The future management of obesity-induced fatty liver disease is likely to move toward precision medicine, integrating genetic, metabolic, and microbiome data to tailor individualized treatment strategies.

9. Therapeutic Innovations and Translational Research

Beyond lifestyle modification, current research is exploring combination pharmacotherapy targeting multiple disease pathways simultaneously. Agents modulating bile acid signalling, incretin pathways, lipid metabolism, and inflammatory cascades are under active investigation. Bariatric surgery has also demonstrated significant benefits in selected patients by improving metabolic parameters and reversing hepatic steatosis and fibrosis.

Conclusion:

Obesity-induced fatty liver disease represents a growing global health concern and stands at the intersection of metabolic dysfunction, chronic inflammation, and progressive liver injury. Far from being a benign accumulation of hepatic fat, this condition reflects a complex, multisystem disorder driven by adipose tissue dysfunction, insulin resistance, altered lipid metabolism, immune activation, and disruptions of the gut–liver axis. The transition from simple steatosis to steatohepatitis, fibrosis, and advanced liver disease underscores the dynamic and multifactorial nature of disease progression.

Recent advances have shifted the understanding of obesity-induced fatty liver disease from a liver-centric disorder to a systemic metabolic condition, emphasizing the roles of lipotoxic intermediates, mitochondrial dysfunction, immunometabolic pathways, and genetic susceptibility. These insights have not only enhanced mechanistic understanding but have also opened avenues for the identification of novel biomarkers and therapeutic targets. Importantly,

fibrosis stage has emerged as the most critical determinant of clinical outcomes, highlighting the need for early detection and timely intervention.

Lifestyle modification remains the cornerstone of management; however, emerging pharmacological agents and metabolic interventions offer promising adjunctive strategies. The integration of non-invasive diagnostic tools, precision medicine approaches, and combination therapies holds significant potential to improve patient outcomes.

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SUPRAMOLECULAR ARCHITECTURE: FROM MOLECULAR RECOGNITION TO CONTROLLED RELEASE

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Abstract:

Supramolecular chemistry, governed by noncovalent interactions, has emerged as a powerful paradigm in modern drug design. By moving beyond traditional covalent medicinal chemistry, supramolecular architecture enables precise molecular recognition, adaptive binding, self-assembly and stimuli-responsive behaviour. These features are particularly valuable for improving drug selectivity, bioavailability, stability and controlled release. This chapter provides a comprehensive overview of supramolecular principles relevant to drug design, starting from molecular recognition and host-guest chemistry, progressing through self-assembled nanostructures and culminating in advanced systems for targeted and controlled drug delivery. Emphasis is placed on the structure function relationships, biological relevance, and translational challenges of supramolecular drug systems.

Keywords: Supramolecular Chemistry, Host-Guest Chemistry, Drug Design, Self-Assembly, Stimuli-Responsive

Introduction:

The evolution of modern chemical science has been profoundly shaped by the transition from the study of individual molecules to the investigation of organized molecular ensembles, a shift marked by supramolecular chemistry twice receiving the Nobel Prize in chemistry (1,2). Defined as "chemistry beyond the molecule," this field exploits a versatile toolkit of non-covalent interactions including hydrogen bonding, π–π stacking, electrostatic forces, hydrophobic effects and metal coordination to construct dynamic, reversible architectures (3-5). While traditional drug discovery has relied on small molecules designed for specific biological targets, the increasing complexity of disease biology and the limitations of conventional therapy such as poor solubility, off-target toxicity and multidrug resistance demand more sophisticated strategies (6,7). Supramolecular chemistry addresses these challenges by enabling molecular recognition and the self-assembly of functional nanostructures that respond to biological stimuli. These features ensure that therapeutic cargos are protected during circulation, selectively recognized at

disease sites and released in a controlled manner (8). Consequently, supramolecular architecture serves as a vital bridge between fundamental molecular recognition and applied controlled drug release, offering a transformative approach to the future of precision medicine (9,10).

Fundamentals of Supramolecular Chemistry in Drug Design

Noncovalent Interactions

Noncovalent interactions form the physicochemical foundation of supramolecular architectures by dictating molecular association, organization and stability through well-defined energetic and geometric parameters (11). Hydrogen bonding, characterized by directionality and specificity, governs recognition processes by aligning complementary donor-acceptor motifs and contributes significantly to binding enthalpy in host-guest and drug-target complexes. Electrostatic interactions, described by coulombic forces and modulated by dielectric constant and ionic strength, dominate long-range attraction and play a critical role in the complexation of charged drugs with oppositely charged supramolecular hosts or polyelectrolytic carriers. Hydrophobic interactions, driven primarily by entropic gains associated with the release of structured water molecules, are central to the self-assembly of amphiphiles into micelles, vesicles, and bilayers, enabling efficient encapsulation of poorly water-soluble drug molecules. Aromatic interactions, including π-π stacking and cation-π interactions, arise from quadrupole moments and orbital overlap effects, providing stabilization and selectivity in systems involving aromatic pharmaceuticals and macrocyclic receptors. Van der Waals forces, encompassing dispersion and induction interactions, contribute to close-range molecular complementarity and fine-tune binding geometries (12). Importantly, the cumulative and cooperative nature of these weak interactions results in high overall binding affinities while retaining reversibility, allowing supramolecular drug systems to respond dynamically to changes in pH, redox potential, enzymatic activity, or competitive binding environments. Such chemically programmable noncovalent interactions underpin structure property relationships in supramolecular drug design, directly influencing drug loading efficiency, thermodynamic stability, and controlled release behaviour. The stability and functionality of supramolecular drug systems arise from a balance of weak, reversible interactions (13). Hydrogen bonding plays a central role in biomolecular recognition mimicking interactions found in DNA base pairing and protein-ligand binding. Electrostatic interactions govern complexation between charged drugs and carriers, while hydrophobic effects drive the assembly of amphiphilic molecules in aqueous environments. Π-π stacking and van der Waals forces further contribute to structural integrity and specificity.

Dynamic and Adaptive Behaviour

Dynamic and adaptive behaviour is an intrinsic characteristic of supramolecular systems arising from the reversible nature of noncovalent interactions and their sensitivity to environmental parameters (14). Because supramolecular assemblies are maintained by weak, kinetically labile

forces, they exist in a dynamic equilibrium in which association and dissociation processes continuously occur. This dynamic exchange allows assemblies to reorganize their structure, composition, and binding modes in response to changes in pH, ionic strength, temperature, redox potential, or the presence of competitive guests (15). From a thermodynamic perspective, adaptive behaviour reflects the minimization of Gibbs free energy through subtle modulation of enthalpic contributions from intermolecular interactions and entropic gains associated with molecular mobility and solvent reorganization. In drug design, such adaptability enables error correction during self-assembly, enhances binding selectivity through induced-fit or conformational selection mechanisms, and permits reversible encapsulation of drug molecules. Dynamic supramolecular systems can thus respond selectively to pathological microenvironments, undergoing structural transitions that trigger drug release while remaining stable under normal physiological conditions (16). This capacity for stimuli responsive reconfiguration, coupled with self-healing and component exchange, distinguishes supramolecular architectures from static covalent systems and underpins their utility in developing smart, controllable, and biologically compatible drug delivery platforms (17).

Molecular Recognition: The Basis of Supramolecular Drug Design

Host-Guest Chemistry

Host-guest chemistry in drug design is distinguished by the use of structurally preorganized molecular hosts that provide confined binding environments for drug molecules, enabling spatial control over molecular recognition. Macrocyclic hosts such as cyclodextrins, calixarenes, cucurbiturils, and pillararenes offer cavities with defined geometry, polarity, and functional group orientation, allowing selective inclusion of guest drugs based on steric complementarity and cavity microenvironment rather than generalized intermolecular attraction (18). This confinement effect leads to predictable structure-property relationships, where subtle changes in host size or functionalization directly influence binding stoichiometry, orientation, and residence time of the encapsulated drug. Encapsulation can shield labile functional groups from hydrolysis, photodegradation, or premature metabolism, while also regulating diffusion controlled release through guest exchange kinetics. Importantly, host-guest systems enable modular formulation strategies in which drug performance can be optimized without chemical derivatization, making them particularly valuable for tuning solubility, stability, and delivery profiles of existing therapeutics within supramolecular drug architectures (19).

Cyclodextrins, for example, have been widely used in pharmaceutical formulations due to their biocompatibility and regulatory acceptance (20). Cucurbiturils offer exceptionally strong binding affinities, enabling high selectivity and sustained drug release (21).

Biomolecular Recognition

Biomolecular recognition in supramolecular drug design exploits the inherent selectivity of biological motifs to achieve highly specific interactions with complex cellular targets. Unlike synthetic host-guest systems that rely on preorganized cavities, biomolecular recognition is governed by sequence-encoded information in peptides, nucleic acids, carbohydrates, and protein derived domains, enabling discrimination between closely related biomolecules (22). Peptide ligands can be designed to recognize receptor binding pockets through secondary structure formation, while nucleic acid aptamers adopt defined three-dimensional folds that bind proteins with antibody-like affinity. Carbohydrate-based recognition elements selectively interact with lectins and cell-surface glycoconjugates, providing access to tissue and cell specific targeting pathways. In supramolecular architectures, these biomolecular motifs function as programmable recognition units that translate molecular level specificity into macroscopic outcomes such as targeted accumulation, cellular internalization, or subcellular localization (23). By integrating biomolecular recognition into supramolecular drug systems, it becomes possible to navigate the complexity of biological environments with precision that cannot be achieved through nonspecific physicochemical interactions alone (24).

Supramolecular Self-Assembly for Drug Delivery

Self-Assembled Nanostructures

Supramolecular self-assembly provides a hierarchical design strategy in which molecular-scale interactions are translated into organized nanostructures that dictate macroscopic drug delivery performance (25). Rather than functioning as passive carriers, self-assembled systems act as chemically programmable entities whose size, morphology, and stability are governed by assembly pathways and environmental conditions. This hierarchical organization allows drug delivery behaviours such as circulation lifetime, cellular uptake, and intracellular trafficking to be encoded during molecular design (26). Importantly, self-assembly enables adaptive responses to biological complexity, allowing drug carriers to maintain structural integrity during transport while retaining the capacity to reorganize at the site of action. Such systems exemplify how bottom-up supramolecular design can bridge molecular chemistry with therapeutic function.

Supramolecular Hydrogels

Supramolecular hydrogels, formed through noncovalent crosslinking, provide three-dimensional networks capable of localized and sustained drug release. Their injectability, self-healing properties, and responsiveness make them suitable for tissue engineering and localized therapy (27).

Targeted Drug Delivery via Supramolecular Architecture

Supramolecular architectures introduce a level of control over targeting that extends beyond simple ligand receptor recognition, emphasizing cooperative and collective effects arising from

multicomponent organization (28). By presenting recognition elements in a spatially controlled manner, supramolecular systems can amplify weak individual interactions into high overall selectivity and avidity. This spatial organization also allows targeting efficiency to be decoupled from drug loading, enabling independent optimization of each function. Furthermore, supramolecular targeting strategies are inherently adaptable, as recognition elements can be exchanged, masked, or activated in response to environmental cues (29). Such architectural flexibility supports the development of delivery systems capable of navigating heterogeneous and evolving disease microenvironments.

Targeting strategies in supramolecular drug design rely on both passive and active mechanisms. Passive targeting exploits the enhanced permeability and retention effect in tumours, while active targeting uses ligands such as peptides, sugars, or antibodies to recognize specific cellular receptors. Supramolecular systems allow multivalent presentation of targeting ligands, enhancing binding affinity through cooperative effects. Additionally, the modular nature of supramolecular assembly enables simultaneous incorporation of targeting, imaging, and therapeutic components (30).

Controlled and Stimuli-Responsive Drug Release

pH Responsive Systems

Many pathological environments, such as tumors and inflamed tissues, exhibit acidic pH. Supramolecular assemblies sensitive to pH changes can disassemble or alter binding strength under acidic conditions, triggering drug release (31).

Redox and Enzyme Responsive Systems

Redox gradients and overexpressed enzymes in diseased tissues provide additional triggers for controlled release. Supramolecular systems incorporating redox-sensitive motifs or enzyme-cleavable components offer high selectivity and minimal systemic toxicity (32).

External Stimuli

Light, temperature, and magnetic fields can also be used to control drug release from supramolecular architectures, enabling spatiotemporal precision in therapy (33).

Translational Challenges and Safety Considerations

The successful translation of supramolecular drug systems requires reconciling chemical elegance with practical constraints imposed by manufacturing, regulation, and clinical use. Complexity at the supramolecular level can complicate reproducibility and scalability, making rigorous control over assembly processes essential (34). Additionally, dynamic behaviours that is advantageous for function must be carefully balanced against requirements for in vivo stability and predictable pharmacokinetics. Comprehensive safety assessment must therefore consider not only the molecular components but also their assembled states, degradation pathways, and long-

term biological interactions. Addressing these challenges will require close integration of supramolecular chemistry with pharmaceutical sciences and regulatory frameworks.

Future Perspectives

Future advances in supramolecular drug design are likely to arise from the convergence of chemical design principles with data driven and biological approaches. The incorporation of predictive modeling, artificial intelligence, and systems biology can accelerate the rational optimization of supramolecular architectures by correlating structural parameters with biological outcomes. Moreover, adaptive supramolecular systems capable of sensing and responding to patient-specific biochemical signatures hold promise for personalized therapeutic interventions (35). Such developments position supramolecular chemistry not merely as a delivery strategy, but as a foundational framework for next-generation precision medicine.

Conclusions:

Supramolecular architecture provides a unifying framework that connects molecular recognition with controlled drug release. By harnessing reversible noncovalent interactions, supramolecular drug systems offer enhanced selectivity, adaptability, and therapeutic efficacy. Continued interdisciplinary efforts will be crucial for translating these elegant chemical concepts into clinically viable medicines.

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OBESITY: A SOCIAL CONTAGION

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1. Introduction:

Obesity is a socially constructed phenomenon that is difficult to define because it is influenced by numerous discourses and constructions from diverse viewpoints (e.g, academia, activism, public health, and medicine). Excess body fat is a defining feature of the complicated condition known as obesity (Kopelman, 2000). Being obese is more than simply a cosmetic issue. This illness increases the risk of developing several other illnesses and health problems. By analysing data from longitudinal studies that link a certain weight to future negative health impacts, the categories of obesity and overweight have been established. Body mass index, or BMI, is the currently recognised proxy for body fatness. It is calculated by dividing weight in kilogrammes by height in meters squared. Underweight individuals have a BMI of less than 18.5 kg/m^2, healthy weight adults have a BMI of 18.5 to 24.9 kg/m^2, overweight adults have a BMI of 25.0 to 29.9 kg/m^2, and obese adults have a BMI of ≥30 kg/m^2. A BMI of 85-95 percentile for age and sex indicates that a child or teenager is at risk of being overweight, while a BMI of 95 percentile or above indicates that the child or adolescent is overweight or obese. Furthermore, not all ethnic groups have comparable absolute or relative metabolic hazards when compared to the traditional cut-points for adult overweight (BMI ≥25 kg/m^2) and obesity (BMI ≥30 kg/m^2). The body mass index (BMI), which is equivalent to weight/height 2 in kg/m2, is the most used way to categorise weight status and illness risk. Women have greater body fat than men at the same BMI. Additionally, the hazards of obesity may be influenced by regional fat distribution. An increased risk of metabolic syndrome, diabetes mellitus, hyperandrogenism in women, heart disease, high

blood pressure, high cholesterol, liver disease, sleep apnoea, and some types of cancer is independently linked to central (mainly visceral) obesity (waist-to-hip ratio, >0.9 in women and 1.0 in men) (Shanget *et al.*, 2021, Kotsis *et al*., 2010) (Figure 1).

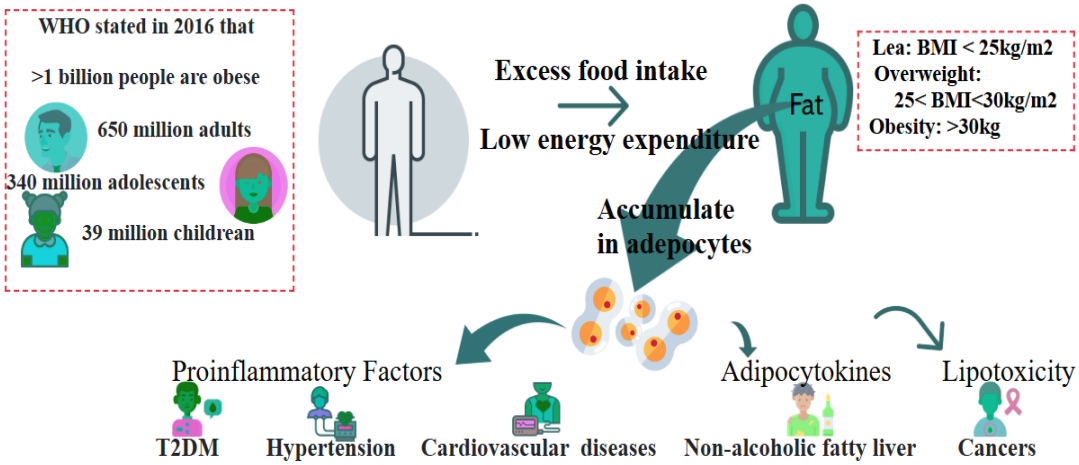

Figure 1: Overview of the obesity epidemic, obesity definition, and obesity-associated diseases (Kotsis *et al*., 2010).

Prevalence of Obesity

The incidence of obesity is rising quickly in many developed countries, making it a serious public health concern both domestically and abroad (Pearce *et al*.,2010). The United States has a 19.7% obesity rate among children and adolescents aged 2 to 19 between 2017 and March 2020, according to the National Health and Nutrition Examination Survey (NHANES) (Stierman *et al*.,2021). According to data from the Centres for Disease Control and Prevention (CDC), 23 US states had adult obesity rates of 35% or more in 2023, while all states had rates of at least 20%. Prior to 2013, no state had a 35% adult obesity rate (Danielson *et al.,* 2025). Over 890 million adults were living with obesity in 2022, out of the 2.5 billion adults aged 18 and over who were overweight. Compared to 1990, when 25% of adults aged 18 and over were overweight, this represents 43% of adults aged 18 and over who were overweight (43% of males and 44% of women). Overweight prevalence varies by area, ranging from 67% in the area of the Americas to 31% in the WHO South-East Asia and African Regions. In 2022, 16% of adults globally who were 18 years of age or older were obese. Between 1990 and 2022, the prevalence of obesity more than quadrupled globally. An estimated 35 million children under five were overweight in 2024. Overweight was always thought to be an issue in high-income nations, but it is now becoming more prevalent in low- and middle-income countries. Since 2000, there has been an approximately 12.1% increase in the number of overweight children under five in Africa. In 2024, Asia accounted for about half of all children under five who were overweight or obese. In 2022, more than 390 million kids and teenagers between the ages of 5 and 19 were overweight. Compared to 1990, when just 2% of children and adolescents aged 5 to 19 were fat (31 million

young people), by 2022, 8% of children and adolescents (160 million young people) were living with obesity (Nam *et al*., 2021).

Pathogenesis of Obesity

The primary cause of obesity is an imbalance between energy expenditure and calorie intake (Gadde *et al*., 2018). Overconsumption of energy will be stored in organs and subcutaneous adipose tissue (SAT) as fat and glycogen (Haas *et al*., 2012, Belaj and Eller, 2012).

The primary cause of obesity is an imbalance between energy expenditure and calorie intake (Gadde *et al*.,2018). Overconsumption of energy will be stored in organs and subcutaneous adipose tissue (SAT) as fat and glycogen (Haas *et al*., 2012, Belaj and Eller, 2012). The depots that make up adipose tissue (AT) have different functions (Ibrahim,2010). While brown adipose tissue (BAT) generates heat in response to β-adrenergic stimulation or cold exposure, a process known as adaptive thermogenesis, white adipose tissue (WAT) is an active endocrine and a significant and safe lipid storage organ (Gaspar *et al*., 2021). Visceral WAT (VAT) and SAT are the two primary depots of WAT in humans, and they have both been extensively researched for their links to the emergence of associated disorders (Reyes *et al.,* 2021). BAT represents merely 1%–2% of fat, but it is vital in maintaining homeostasis and shows beneficial effects on blood glucose (Becher *et al*.,2021). Overweight and obese individuals have been associated with a low-grade, chronic inflammatory state, which is connected to increased infiltration of M1 or "classically activated" macrophages from the circulation into AT (Coenen *et al*., 2007). TNF-α, IL-6, IL-8, and other inflammatory cytokines are secreted by these macrophages when they are attracted to AT (Reilly and Saltiel, 2017). Adipocytes secrete anti-inflammatory cytokines (like IL-4, IL-10, IL-13, and IL-19) in addition to pro-inflammatory cytokines; however, their secretion and abundance seem to decline with weight gain because obesity unquestionably causes the balance to increase the production of more pro-inflammatory adipokines (Guzik *et al*., 2017, Kawai *et al.,* 2021). To control associated pathways, AT also secretes extracellular matrix (ECM) components and adipokines, including resistin, visfatin, adiponectin, and leptin (Hermano *et al*., 2019, Galic *et al.,* 2010).

As adiponectin production declines, leptin, one of the most prevalent adipokines with proinflammatory qualities, rises in tandem with other factors like hepatocyte growth factor (HGF) (Bell *et al*.,2006), plasminogen activator inhibitor-1 (PAI-1), resistin (Askarpour *et al*.,2020), TNF-α, IL-1β, IL-6, and monocyte-chemo attractant protein-1 (MCP-1) (Cava and Matarese,2021, Obradovic *et al*.,2021). By upregulating PPARγ, which is linked to tumour growth and initiation through cell intrinsic and extrinsic mechanisms (Ha *et al*.,2020), elevated free fatty acids (FFAs) in the serum of obese people increase the expression of vascular endothelial growth factor-A (VEGF-A) and vimentin, leading to insulin resistance (Boden, 2011, Neuschwander, 2010) and hepatic steatosis (Neuschwander, 2010). Insulin resistance in muscle,

liver, islet α-cells, and AT can be brought on by overexpression of TNF-α and leptin, which can also prevent insulin receptor activation and ultimately result in type 2 diabetes (Niswender, 2010).

Pathomechanism of Obesity

According to twin and family studies, the percentage of BMI heredity is rather high, ranging from 40 to 70 percent (Bray *et al*., 2016, Elks *et al*., 2012). More than 300 loci with common variations in the general population have been found by large-scale genome-wide association studies, and these loci significantly correlate with obesity features (Gonzalez *et al*.,2017). Less than 5% of the variance in BMI can be explained by these loci, which have relatively little impact on the risk of obesity (Winkler *et al*., 2015, Locke *et al*., 2015).

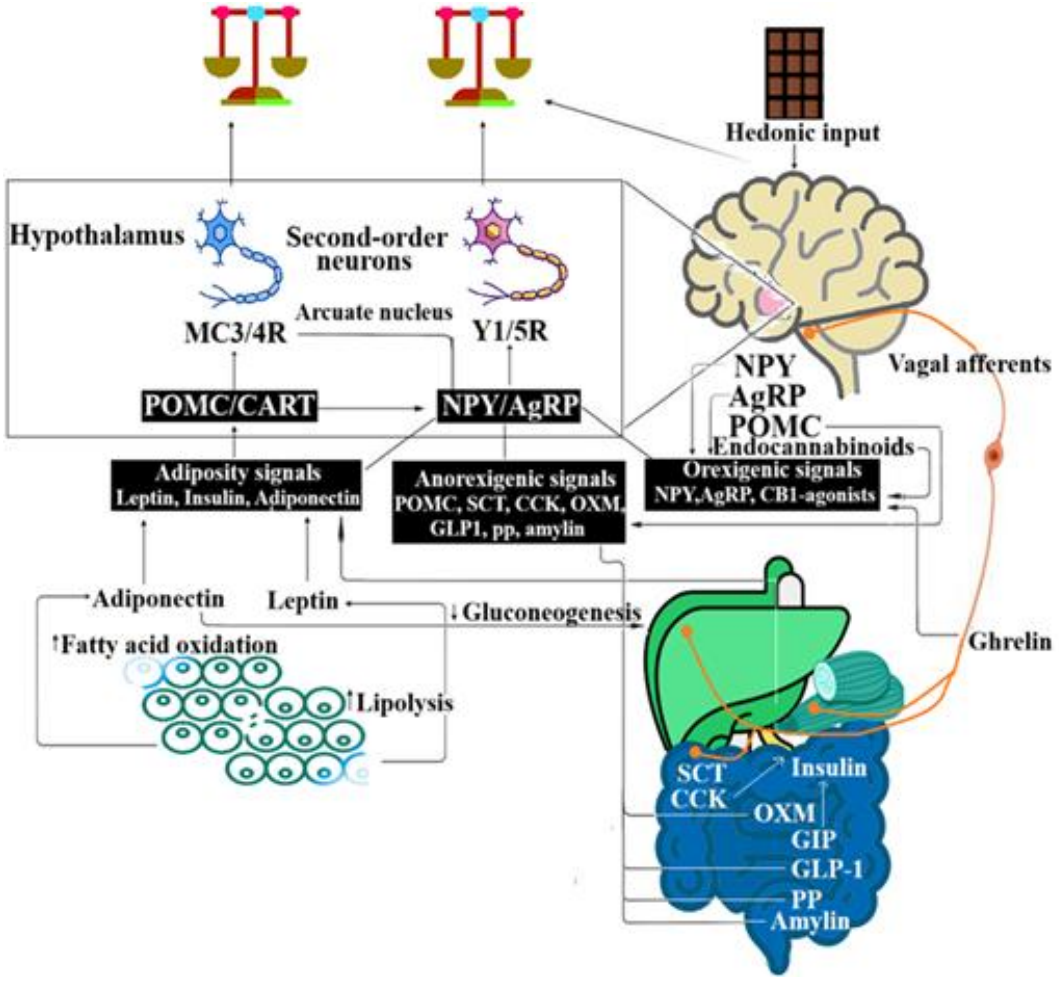

Figure 2: Energy balance signals integration (Hetherington and Ranson, 1940)

In the pink-yellow quadrant, there is a simplified representation of hypothalamic energy balance regulation mechanisms: primary neurons in the arcuate nucleus include appetite-inhibiting neurons (blue)–cocaine-and amphetamine-stimulated transcript peptide (CART) and proopiomelanocortin (POMC), which release peptides that stimulate the melanocortin receptors (MC3 and MC4). MC3/4R stimulation increases energy expenditure and decreases appetite. This circuit is stimulated by adiposity and anorexigenic signals. Peripheral signals related to long-

term energy stores are produced by adipose tissue (leptin, adiponectin) and the pancreas (insulin). Gut hormones with incretin-, hunger-, and satiety-stimulating effects: glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and potentially oxyntomodulin (OXM) improve the response of the endocrine pancreas to absorbed nutrients; GLP-1 and OXM also centrally reduce food intake; secretin (SCT) and cholecystokinin (CCK) released from the gut inhibit appetite by way of the vagus nerves, which stimulate hindbrain structures.

Diagnosis for Clinical and Preclinical obesity

Both more sophisticated diagnostic tools and conventional anthropometric measures can be used to evaluate obesity.

1. Traditional Measurements of Obesity

Although they might not accurately depict the distribution of body fat, they are frequently utilised since they are easy, rapid, and affordable (Table 2).

2. New / Advanced Diagnostic Methods

These aim to more accurately quantify visceral fat, body fat percentage, and fat distribution (Table 3).

Table 1: Conventional Methods of Assessing Obesity

Method	Measures	Common Cut-offs	Pros	Limitations
BMI = weight (kg) ÷height² (m^2)	General classification of weight status	Overweight: BMI ≥ 25; Obese: BMI ≥ 30	Easy to calculate; widely recognized	Does not distinguish between muscle and fat; ignores fat distribution
WC	Abdominal fat	Risk ↑ if >102 cm (men), >88 cm (women)	Quick and low-cost; reflects central obesity	Can vary with posture, breathing
WHR = waist ÷ hip circumference	Fat distribution (visceral vs subcutaneous)	Men: >0.90; Women: >0.85 → higher risk	Simple; better indicator of cardiometabolic risk than BMI	Less used in routine practice
ST (calipers)	Subcutaneous fat at specific sites	Age- and sex-specific tables	Inexpensive; portable	Requires training; less accurate in very obese

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist-to-Hip Ratio; ST: Skinfold Thickness

Table 2: Emerging Diagnostic Approaches

Method	Principle	Advantages	Limitations
BIA	Electrical current estimates body fat & lean mass	Quick, portable; gives % body fat	Accuracy affected by hydration status
DEXA	X-ray beams differentiate bone, lean mass, and fat	Highly accurate; measures regional fat	Expensive; limited availability
MRI / CT Scan	Imaging to quantify visceral and subcutaneous fat	Gold standard for fat distribution	Very costly; not for routine screening
Bod Pod	Measures body volume to estimate fat	Accurate; non-invasive	Requires special equipment
Ultrasound	Measures subcutaneous fat thickness	Radiation free; bedside use	Operator dependent

BIA: Bioelectrical Impedance Analysis; DEXA: Dual-Energy X-Ray Absorptiometry; Bod Pod: Air Displacement Plethysmography.

Pharmacotherapy

When combined, pharmacotherapy and lifestyle modifications result in cumulative weight loss. Maintaining weight loss may also be made easier with pharmacotherapy combined with lifestyle changes (Apovian *et al*., 2015, Garvey *et al*, 2016, Yanovski and Yanovski, 2014). The Food and Drug Administration (FDA) authorised the inexpensive sympathomimetic amine phentermine for short-term (≤3 months) usage in 1959, making it the most often prescribed weight-management drug in the United States (Apovian *et al*., 2015). Some professional associations have discouraged long-term use of phentermine due to the availability of five newer FDA-approved weight-management medicines and the complications associated with administering it (Apovian *et al*., 2015, Garvey *et al*, 2016, Yanovski and Yanovski, 2014). The FDA needs at least a year of studies to demonstrate the drug's safety and a mean difference in weight reduction of 5% or more between the treatment group and the placebo group before approving a new weight-loss medicine. As an alternative, at least 35% of individuals in the treatment group must lose 5% or more of their baseline weight, which is almost twice as many as those in the placebo group (Yanovski and Yanovski, 2014). Three single meds and two combination medications make up the five authorised treatments for long-term weight control. Table 4, Figure 3 summarises the key characteristics of these medications, which are usually used in conjunction with low-to-moderate-intensity lifestyle coaching (≤1 session per month).

Table 3: Anti-obesity drugs and their mechanism of action

Drug	MOA	Dose	MWL	Side Effects	Contraindications
Orlistat	Pancreatic and gastric Lipase inhibitor; Resulting fat malabsorption reduces net energy intake	120 mg	Drug, 8.8 (8.8); placebo,5.8 (5.8); PSWL, 2.6	Oily spotting, flatus with discharge, fecal urgency, oily evacuation, increased defecation, fecal incontinence	Pregnancy, chronic malabsorption syndrome, cholestasis (Davidson *et al*.,1999)
*Lorcaserin	Selective 5HT2C receptor agonist; promotes satiety to reduce food intake	10 mg	Drug, 5.8 (5.8); placebo,2.2(2.2); PSWL,3.2	In patients without diabetes: headache, dizziness, fatigue, nausea, dry mouth, constipation; in patients with diabetes: hypoglycemia, headache, back pain, fatigue	Pregnancy (Smith *et al*.,2010)
Liraglutide	GLP-1 agonist; delays gastric emptying to reduce food intake	Start dose, 0.6 mg (S.c); Dose increased/ wk by 0.6 mg as tolerated to reach 3.0 mg	Drug, 8.4 (8.0); placebo, 2.8 (2.6); PSWL, 5.3	Nausea, vomiting, constipation, headache, hypoglycemia, diarrhea, abdominal pain, fatigue, dizziness, increased lipase levels	Pregnancy, personal or family history of medullary thyroid cancer, or multiple endocrine neoplasia type 2 (Pi-Sunyer *et al*.,2015)

Phentermine and Topiramate	Norepinephrine-releasing agent (phentermine), GABA receptor modulation (topiramate); decreases appetite to reduce food intake	Start dose, 3.75 mg/23 mg for 2 wk; recommended dose,7.5 mg/ 46 mg; maximum dose,15 mg/ 92 mg	Drug, 8.1 (7.8) at recommended dose, 10.2 (9.8) at maximum dose; placebo, 1.4 (1.2); PSWL, 8.8	Insomnia, dry mouth, constipation, paresthesia, dizziness, dysgeusia	Pregnancy, glaucoma, hyperthyroidism, MAOIs, hypersensitivity to sympathomimetic Amines (Gadde *et al*.,2011)
Naltrexone /bupropion	Opioid antagonist (naltrexone), dopamine and norepinephrine reuptake inhibitor (bupropion); acts on CNS pathways to reduce food intake	1 tablet (8 mg of naltrexone and 90 mg of bupropion) daily for 1 wk; dose subsequently Increase each wk by 1 tablet per day	Drug, 6.2 (6.4); placebo, 1.3 (1.2); PSWL, 5.0	Nausea, constipation, headache, vomiting, dizziness, insomnia, dry mouth, diarrhea	Uncontrolled hypertension, Seizure disorders, anorexia nervosa or bulimia, drug or alcohol withdrawal, use of MAOIs, long-term opioid use, pregnancy (Apovian *et al*.,2013)

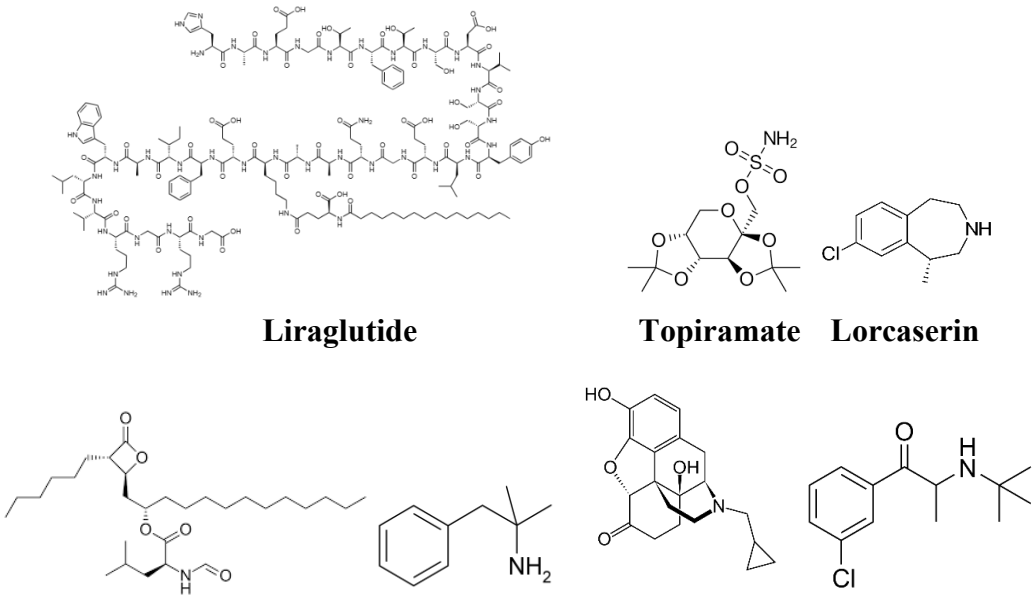

Figure 3: Structure of anti-obesity drugs

Conclusion:

Adipose tissue targeting directly is still a long way from becoming a panacea for type 2 diabetes and obesity. Targeting BAT to raise energy expenditure, however, opens up new possibilities. However, before targeting BAT as a treatment option becomes feasible, a number of problems need to be addressed. It is currently unclear if long-term BAT activation results in enough energy expenditure to meet the weight loss treatment objective. Whether compensatory mechanisms like increased appetite could offset higher energy expenditure is also unknown. The physiological stimulation that seems to activate BAT the greatest is well-directed cold exposure. More research is required to determine the ideal circumstances for a focused cold exposure of a planned "cold-therapy." Many druggable targets have been found thus far, and a pharmacological and cell-based therapeutic approach appears to be a viable option. However, a number of safety issues still exist, necessitating the development of novel therapeutic targets and compounds in accordance with a thorough benefit-risk analysis.

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PREPARATION OF PEG, PVA AND CTAB MEDIATED NANO $CaCO_3$ FROM DOLOMITE ROCK BY BIOMIMETIC METHOD

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Abstract:

Nanoscience and nanotechnology have emerged as rapidly evolving multidisciplinary fields due to the unique physicochemical properties exhibited by materials at the nanoscale (1–100 nm). Among various nanomaterials, nano-sized calcium carbonate ($CaCO_3$) has attracted considerable attention because of its low cost, environmental friendliness, biocompatibility, and wide industrial applicability. The present study focuses on the synthesis of polymer-mediated nano $CaCO_3$ nanocomposites using a natural carbonate source, dolomite, collected from Salem district, Tamil Nadu, India. Polyethylene glycol (PEG), polyvinyl alcohol (PVA), and cetyltrimethylammonium bromide (CTAB) were employed as structure-directing agents to control particle growth and crystallinity. Structural and functional characterizations were carried out using FTIR and XRD techniques. The results confirm the formation of high-purity rhombohedral calcite without any secondary impurity phases. Among the polymer-assisted samples, PEG-mediated nano $CaCO_3$ exhibited the smallest crystallite size due to effective steric hindrance and controlled nucleation. Further optimization of PEG concentration revealed that 0.005 mol PEG-assisted nano $CaCO_3$ possessed superior structural characteristics. The synthesized nano $CaCO_3$ demonstrates significant potential for applications in biomedical, food, cosmetic, industrial, and energy storage sectors, highlighting the importance of polymer-mediated approaches for tailoring nanocomposite properties.

1. Introduction:

Nanoscience is the study of objects/particles that are 1 to 100 nanometers in size. Nanoscience is the study of the basic link between physical qualities and material dimensions at the nanoscale. Nanoparticles are particles of 100 to 10,000 atoms. They are the fundamental components of nanomaterials. Nano is a scientific unit that represents one billionth of a base unit. The term 'scale' refers to the magnitude of an object's size or length in nanometers. Nanoparticles possess

structural, optical and electrical features that individual molecules or bulk materials are short of (1, 2).

Nanotechnology is concerned with the fundamental study of nanomaterial and nanostructure physical properties and phenomena. Materials and systems developed with nanotechnology have innovative and greatly improved physical, chemical, and biological qualities. The ability to manufacture highly ordered nanoparticles of any size and form is a recent advancement in nanotechnology that has led to the invention of new biocidal agents. It is a multidisciplinary scientific field that is rapidly evolving.

This technology has applications in diagnostics, medicine delivery, sunscreens, gasification, hydrogenation, pyrolysis, antimicrobial bandages, disinfectants and a waste-reduction manufacturing method. Additionally, it is employed to make a substance that increases the effectiveness of the present preparing techniques by reducing or avoiding the use of hazardous materials to reduce pollution and producing alternative energy (3).

Improvement of charge separation, life time of charge carrier, It can be achieved interfacial charge transfer efficiency. Increase the photocatalytic performance avoids agglomeration and photo corrosion Charge recombination could be significantly reduced and high surface area could be maintained.

1.2 Types of Nanocomposites

- Metal oxide based nanocomposites
- Polymer – based nanocomposites
- Carbon-based nanocomposites

1.3 Types of Minerals

According to Blyth (4) and Wadia (5), many thousands of minerals have been documented so far, but only about thirty are most common. Natural elements like sulphides and oxides are basic types of minerals. The minerals found in the Earth's crust that are most extensively dispersed are carbonates, followed by sulphates, carbonates, phosphates, halides, silicates, etc., any class of minerals in which the fundamental structural and compositional unit is the carbonate ion CO_3^{2-}.

Minerals are generated naturally by geological processes and include the chemical makeup of the material as well as the structure of the mineral. Minerals come in a variety of physical and chemical forms, including crystalline, amorphous, massive, earthy and so on, depending on their method of genesis and molecular structure.

1.4 Uses of Minerals

A mineral is a naturally occurring material that has unique composition, atomic structure and chemical and physical characteristics. Minerals, metals, rocks and hydrocarbons (solid and liquid) that are extracted from earth's crust by mining, quarrying and pumping are all included in

the greater definition of an economic mineral. Applications for economic minerals are numerous and include industry, energy production, building and agriculture.

- Energy minerals are used in the production of polymers, electricity, fuel for vehicles and heating for individuals and companies. Uranium, oil, coal and natural gas are examples of energy minerals.
- Some metals are used in vehicles and building frames (iron as steel), in electrical wiring (copper), in rechargeable batteries (lithium) and in aircraft and drink container production (aluminum). Mobile phones and jewelry are made of precious metals.
- Sand, gravel, brick clay, and crushed rock aggregates are examples of construction materials that are used to make concrete, bricks, pipes and to build roads and homes.
- Non-metallic minerals, also referred to as industrial minerals, can be used in a variety of industrial processes, such as the production of chemicals, glass, plastics, paper, fertilizers and fillers for medications. Salt, clays, graphite, limestone, silica sand, phosphate rock, talc and mica are examples of industrial minerals.
- Exploring mineral resources suitable for economic exploitation is the goal of mineral exploration. Numerous techniques, including as geochemical surveys and remote sensing, may be employed.

For the advancement of technology, the economy and society, minerals are essential in their natural resources.

1.5 Calcium Carbonate Minerals ($CaCO_3$)

Calcium carbonate ($CaCO_3$) is also one of the most researched biominerals and it has been derived from a variety of living things, including seashells, coral and sea urchin spines. Because of its beneficial properties (structural, optical and surface), synthesised $CaCO_3$ can be used in a variety of applications, including filler materials, paints, plastics, paper, textile, rubber, sealants, cosmetics, toothpaste, foodstuffs, photocatalyst, biosensor, medicine, pharmaceutical industry and drug delivery systems (6-8).

The dolomite mineral can be used as source of calcium. These sources are easy to get in lower prices with lower toxicity. If these sources are used in some materials, the product will have excellent fill volume, brightness and the integrating character of good processing and performance.

Even carbonate minerals have some enormous applications, some of the properties such as hydrophilic nature, low surface area and production process are not able to get all the application. In order to enhance the properties, the materials need to convert into nano size.

Compared to bulk calcium carbonate, nanosized calcium carbonate exhibits more benefits and unique properties, such as a much higher specific surface area and a tendency to blend together (9). In the modern era, the research is focused in nano calcium carbonate.

1.6 Importance of Nano Calcium Carbonate

Ultra-fine nano calcium carbonate particles have superior the quantum size effect, magnetic, catalytic, light resistance, surface effect, small size effect and melting point.

Modern researchers have been paying significant focus on nano $CaCO_3$ in recent years as low-cost, commercially available alternative filler for a variety of industrial applications. From an environmental perspective, it is also good filler because of its low toxicity, low pollution and white color (10).

1.7 Applications of Nano $CaCO_3$

1.7.1 Medical

Nano $CaCO_3$ is most useful in drug delivery in biomedical field. Biominerals or additives like calcium carbonate ($CaCO_3$), calcium phosphate ($CaPO_4$) and hydroxyapatite ($Ca_5(PO_4)_3(OH)$) might be promising materials in biomedical applications, primarily in bone tissue regeneration (11). There are several applications for nano calcium carbonate ($CaCO_3$) in the food supplement, cytotoxicity, intestinal transit, cellular uptake and oral absorption domains.

1.7.2 Food

In food processing, fewer stabilizers are used to achieve equivalent suspension, or to improve the suspension with the current level of stabilizer using nano precipitated calcium carbonate. It is also used in food beverages.

1.7.3 Cosmetics and Toiletries

Nano precipitated calcium carbonate play roles in formulating outstanding toiletries, cosmetics and other products for daily use such as, medicated powders, toothpastes, creams and lotions, pressed, gels and loose face powders and liquid preparations, solid stick deodorants, eye shadows and dry shampoo.

1.7.4 Industrial Material

Application of nano calcium carbonate is growing in the industries. It has the ability to modify the performance characteristics of materials including adhesives, sealants, rubber and plastics. These characteristics include improved impact heat resistance, superior toughness, weather resistance and performance are possible under extreme temperature conditions by nano $CaCO_3$. It is used to replace carbon black which is cheap and substitute. This is highly required in rubber and tyre industry. Incorporation of silicon dioxide (5-20 %) with nano precipitated calcium carbonate (NPCC) is used to increase elongation, tensile strength, aging, tear, abrasion resistance without impacting reinforcing features with low cost.

In paint industry, calcium carbonate ($CaCO_3$), iron oxide and carbon black are used as prime pigment for color coating or some bright colored organic pigment with high expensive. Nano $CaCO_3$ is used as a filler mineral to achieve the targeted balance of application (latex or emulsion paints) such as appearance, durability and cost properties.

Typically 20 % NPCC is used to replace the existing additives such as CPE, MBS and SBE in plastic sector. It is used to improve finishing, impact strength (12) and thermal conductivity (13). In printing ink, NPCC is better additive offset and lithographic ink. It can serve to replace oils and varnish. It is also used in coating such as architectural/decorative, automotive, specialty coatings, traffic or road marking, automobile, refinishing and marine and aerosol paints. According to Sunqing *et al.* (14), nano $CaCO_3$ can act as better additives in lubricant oils. It has strong anti-wear, friction-reducing, and load-carrying properties.

1.7.5 Energy Storage

In the past years, calcium batteries are available, but it is not acquiring reversible process. Now a day, calcium anode is used in rechargeable batteries with the reversibility process (15).

1.7.6 Polymers

Polymer chains-three-dimensional networks that constitute the polymer bonds are formed when constituent elements, known as monomers, react with one another during the polymerization process.

Depending on the kind of functional groups bonded to the reactants, a particular polymerization method may be employed. Nearly all macromolecules in the biological setting are either entirely polymeric or composed of long polymeric chains.

A polymer or copolymer with nanoparticles dispersed throughout the polymer matrix that makes up polymer nanocomposites. The group focused on polymer nanotechnology will create methods that facilitate the patterning of functional surfaces. The development of adhesives, sealants, coatings, potting and encapsulating compounds has benefited greatly from the application of nanotechnology. Bentonites, zeolites, and nano-sized silica particles are examples of nanoparticle fillers that have led to the creation of goods with improved tensile strength, transparency, thermal conductivity, water/chemical resistance and thermal stability.

Based on the above discussion, in this work the preparation of various polymers (PEG, PVA and CTAB) mediated nano $CaCO_3$ has been prepared by using dolomite rock as a carbonate materials using biomimetic method.

2. Materials and Methods:

2.1 Raw Materials and Chemicals

Natural carbonate source like Dolomite rock ($CaMg(CO_3)_2$) (approximately 5 Kg) were collected from Arisipalayam village, Salem district, Tamilnadu, India. For synthesis sucrose, anhydrous sodium carbonate, polyethylene glycol (PEG - 600), polyvinyl Alcohol (PVA), CTAB and HCl were purchased from Sigma-Aldrich.

2.2 Preparation of PEG, PVA and CTAB Mediated Nano $CaCO_3$

Synthesis procedure for polymer mediated (PEG, PVA and CTAB) mediated nano $CaCO_3$ were explained in Venkidasamy *et al.,* (16).

3. Results and Discussions:

3.1 FTIR

Fig. 1 (a - c) reveals the FTIR spectrum of PEG, PVA and CTAB mediated nano $CaCO_3$. From these figures, the presences of all major and minor peaks around 1432 (ν_3), 876 (ν_2), 712 (ν_4), 1796 ($\nu_1+\nu_4$) and 2512 ($\nu_1+\nu_3$) depict the presence of calcite (17). The peak observed at 1062 cm^{-1} (Fig. 1(a) and 1085 cm^{-1} (Fig. 1(b)) indicates the presence of PEG and PVA on the surface of nano $CaCO_3$ (18, 19). In the Fig. 1(b), the major peak at 1423 cm^{-1} is slightly broaded and shifted to 1432 cm^{-1}. It clearly shows the effect of PVA on the product surface. In the Fig. 1(c), the intensities of major peaks are slightly increased and wave numbers are shifted when adding the CTAB on the nano $CaCO_3$ (20). The peaks around 3420, 3425 and 3423 cm^{-1} exhibit the presence of OH stretching modes of water or hydroxyls on the product surface (21).

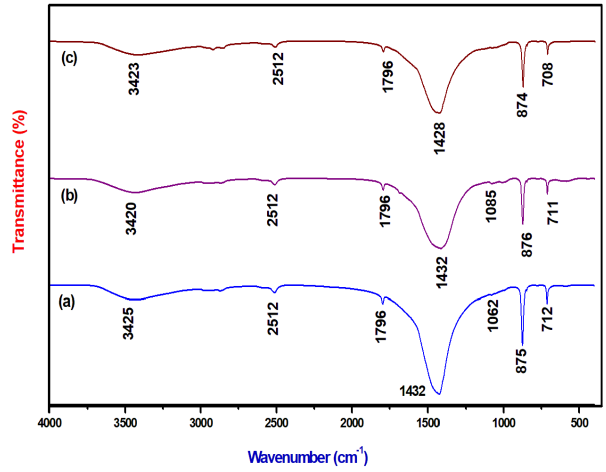

Figure 1: FTIR spectrum of (a) PEG (b) PVA and (c) CTAB mediated $CaCO_3$ nanocomposites

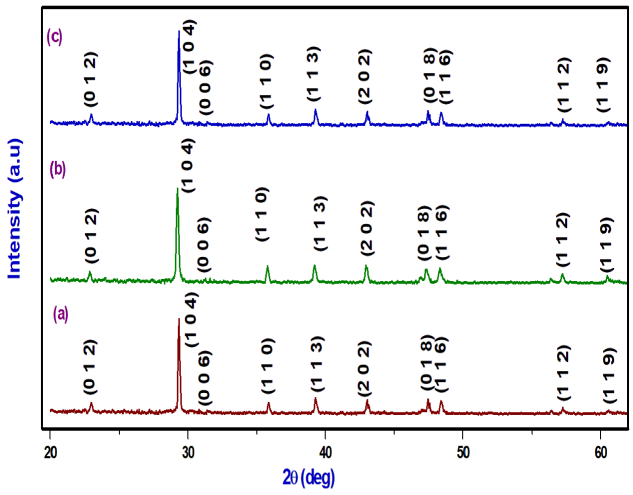

Figure 2: XRD pattern of (a) PEG (b) PVA and (c) CTAB mediated $CaCO_3$ nanocomposites

3.2 XRD

Figure 2 (a-b)) depicts the XRD pattern of PEG, PVA and CTAB mediated $CaCO_3$ nanocomposites. From the figure the obtained various diffraction planes at (0 1 2), (1 0 4), (0 0 6), (1 1 0), (1 1 3), (2 0 2), (0 1 8), (1 1 6), (1 1 2) and (1 1 9) with 22.97º, 29.34º, 31.35º, 35.89º, 39.33º, 43.07º, 47.49º, 48.44º, 57.30º and 60.65º which are well matched with the rhombohdral calcite polymorphs of $CaCO_3$ (JCPDS No: 86 - 2343). It is note worthy to mention that no additional planes other than calcite are observed. While adding the PEG, PVA and CTAB the major planes at 29.34º is slightly shifted to lower angle side. This shows that PEG, PVA and CTAB could be embedded on the product (16).

From the FTIR and XRD analysis, as no impurity peaks are observed, achieved product has high purity.

The crystallite sizes of synthesized PEG, PVA and CTAB mediated $CaCO_3$ nanocomposites were calculated using Scherrer's formula (22).

$$D = \frac{k\lambda}{\beta cos\,\theta}\,(\text{Å})$$

Where D- is the crystallite size (nm), λ is the wavelength of the Cuk_α (1.5406Å), k- constant (0.94), β is the FWHM (full- width half maximum) and is the diffracted angle. The calculated average crystallite size of synthesized PEG, PVA and CTAB mediated $CaCO_3$ nanocomposites were 29, 35 and 36 nm respectively. Among the polymer mediated $CaCO_3$ nanocomposites, the average crystallite size of PEG mediated $CaCO_3$ is lower than others. This result shows the role of polymer to control the size of the products or reducing the nucleation of growth products. In this instance, the growth rate reduces the size of the crystallites which is dominated by the nucleation rate. PEG with more functional groups covers a huge surface area of the nanoparticles with more steric hindrance, which has a detrimental influence on the crystal particles ability to develop (23).

Based on the above discussion, PEG mediated $CaCO_3$ nanocomposites has lower crystallite size than other polymer assisted products. Hence, PEG is taken for further synthesis process.

3.3 FTIR spectra of 0.003, 0.005, 0.007 and 0.009 mol PEG assisted $CaCO_3$ nanocomposites

Fig. 3 (a - d) depicts the FTIR spectra of 0.003, 0.005, 0.007 and 0.009 mol PEG assisted $CaCO_3$ nanocomposites and are represented as nCP_1, nCP_2, nCP_3 and nCP_4. The observed characteristics major and minor peaks in all the cases (Fig. 3 (a – d)) are well matched with the calcite peaks (24). The observed peaks are similar when compared with the observed peaks of Fig. 1(a). Specifically, some visible shift and additional peaks in all the cases such as 1062, 1110, 1112 and 1114 cm^{-1} are observed. It is due to the addition of PEG in the $CaCO_3$ (18, 23). Among the various concentrations (nCP_1, nCP_2, nCP_3 and nCP_4), intensities and broadness of peaks are

comparatively high in nCP_2. The peaks at 3439, 3432, 3420 and 3427 cm^{-1} exhibit the presence of OH stretching **modes of water or hydroxyls (21).**

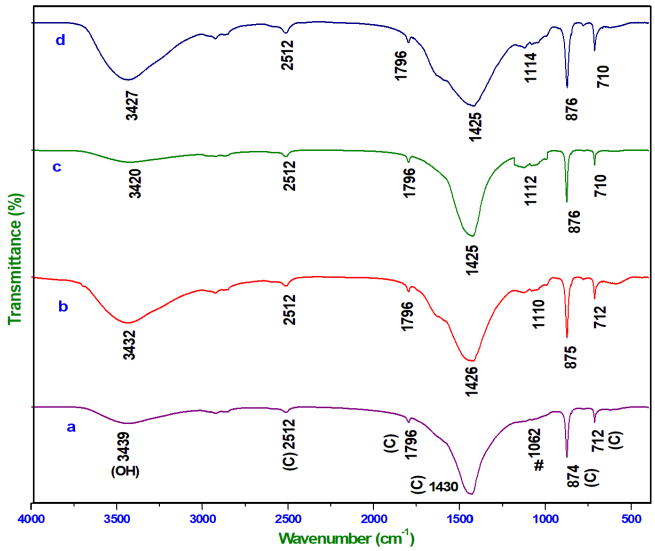

Figure 3: FTIR spectrum of (a) 0.003 (b) 0.005 (c) 0.007 M and (d) 0.009 mol PEG mediated $CaCO_3$ nanocomposites

3.4 XRD pattern of 0.003, 0.005, 0.007 and 0.009 mol PEG assisted $CaCO_3$ nanocomposites

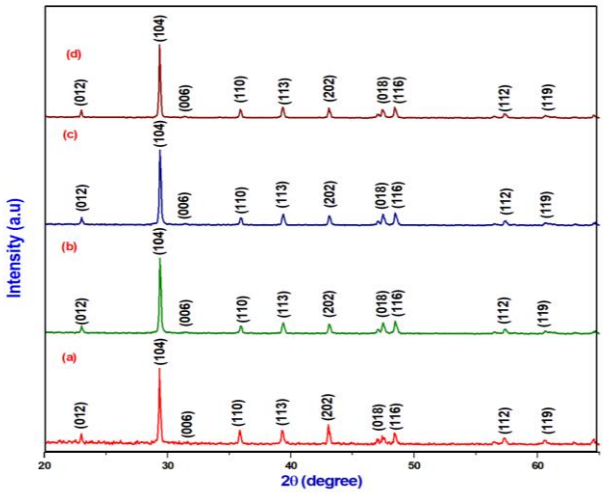

Figure 4: XRD pattern of (a) 0.003 (b) 0.005 (c) 0.007 and (d) 0.009 mol PEG mediated $CaCO_3$ nanocomposites

Fig. 4 (a - d) shows XRD patterns of the various concentrations of PEG loaded $CaCO_3$. All the characteristic planes in all cases are very well matched with the characteristic planes of rhombohedral $CaCO_3$ (JCPD card No: 86 - 2323) as observed in Fig. 2 (a). Due to impact of PEG, the intensity of plane (1 0 4) is increased and there are no any other additional planes are observed (25). Average crystallite sizes are calculated and the crystallite sizes of nCP_1, nCP_2, nCP_3 and nCP_4 are 29, 28, 33 and 36 nm respectively.

The observed results strongly evidenced the possibility of preparation of $CaCO_3$ nanoparticles in a large scale from a natural source (Dolomite rock) by a simple, cost-effective *biomimetic method*. Also, this work witnessed the possibility of various polymer mediated $CaCO_3$ nanocomposites derived from natural dolomite rock.

Conclusion:

In this study, polymer-mediated nano calcium carbonate nanocomposites were successfully synthesized using dolomite as an economical and environmentally benign calcium source. The use of PEG, PVA, and CTAB as mediating agents effectively influenced the crystallite size and surface characteristics of the synthesized nano $CaCO_3$. FTIR and XRD analyses confirmed the formation of phase-pure rhombohedral calcite with no detectable impurities, demonstrating the efficiency of the biomimetic synthesis route. Among the polymers studied, PEG showed superior control over particle growth, resulting in reduced crystallite size due to enhanced steric effects and suppressed crystal agglomeration. Optimization of PEG concentration revealed that 0.005 mol PEG-mediated nano $CaCO_3$ exhibited the most desirable structural features. Owing to its enhanced surface area, low toxicity, and tunable properties, the synthesized nano $CaCO_3$ holds strong promise for diverse applications, including biomedical engineering, food processing, cosmetics, industrial fillers, coatings, and energy storage devices. Overall, this work highlights the potential of polymer-assisted nanotechnology strategies in developing value-added nanomaterials from natural mineral resources for advanced technological applications.

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HOW TO WRITE A RESEARCH AND REVIEWS ARTICLE IN CHEMICAL, BIOLOGICAL AND PHARMACEUTICAL SCIENCE

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Abstract:
In the chemical, biological, and pharmaceutical sciences, research and review articles are written using conventional forms such as IMRaD for original research or a theme synthesis for reviews. These forms, which are exclusive to disciplines like pharmacology and phytochemistry, guarantee clarity, reproducibility, and scientific rigour. Important rules place a strong emphasis on clear abstracts, thorough procedures, and accurate citations. While review articles summarise the body of existing literature without providing new data, research articles report fresh experiments. IMRaD (Introduction, Methods, Results, Discussion) is used in pharmaceutical sciences research publications, and reviews frequently adhere to a similar structured framework with predetermined search methods.

Introduction: Describe the research problem, its importance, and the gap in the literature in one to two pages. Employ a funnel approach: objectives/hypothesis, study outline, specific justification and broad context Cite ten to twenty important references and conclude with a clear goal.

Methods: Describe the replication processes, including the materials (plant extracts, animal models, etc.), procedures, statistical analysis, experimental design (ethanol-induced ulcer, for example), and ethics (IACUC approval, for example). Make use of passive voice and subheadings such as "Histopathology" or "Phytochemical screening".

Result and Discussion: Analyse the findings, contrast them with existing research, discuss the limits, and make recommendations for future research. Restate the main conclusions first, stay away from additional information, and make a connection to the introduction.

Keywords: Research Article, Review Article, Pharmaceutical Sciences and Methodology

Introduction:

The term "Reviews in Chemical, Biological and Pharmaceutical Science" describes academic journals that summarise and assess current research in the related disciplines of biology, chemistry, and pharmaceutical science. These review papers offer thorough summaries of important developments, new trends, and the current level of knowledge on particular subjects rather than presenting fresh experimental data. A review article is a journal article that provides an overview of the state of knowledge on a certain issue within a particular discipline. Since a review article may examine and evaluate the methodology and findings of previously published studies, it is typically regarded as a secondary source.

It resembles a survey article or, in news publishing, overview article, which also surveys and summarizes previously published primary and secondary sources, instead of reporting new facts and results (1,2).

History

- **Research:** Interdisciplinary developments at the nexus of chemistry, biology, and drug development are examined in Research and Reviews in Chemical, Biological, and Pharmaceutical Sciences. An organised summary of important research topics in these areas is given in this sample book chapter.
- **Chemical Sciences:** Organic, inorganic, physical, analytical, and theoretical chemistry, focusing on the evaluation of chemical compounds, their interactions, and the latest advancements in synthesis and analysis.
- **Biological Sciences:** Molecular biology, biochemistry, biotechnology, and pharmacology, covering subjects like the biological activity of medicinal plants, the use of biological creatures in research, and how medications work at the molecular level.
- **Pharmaceutical Sciences:** Clinical trials, design, formulation, delivery methods, quality assurance, drug development, and regulatory affairs. This covers subjects including targeted therapy development, analytical method validation, and process analytical technology (PAT).

About the Research

The fundamental framework of research publications in the chemical, biological, and pharmaceutical sciences is IMRaD (Introduction, Methods, Results, and Discussion), which guarantees a coherent progression from problem to interpretation. Reproducibility and peer review are supported by this format, which has been in use since the 20th century, particularly in pharmacology and phytochemistry investigations such as anti-ulcer research. Research is a methodical process of investigation that uses observation, experimentation, and analysis to find, understand, or modify facts, hypotheses, or applications. It adheres to organised

procedures to guarantee dependability in scientific contexts such as chemical, biological, and pharmaceutical sciences (3).

The Middle French word "recherche" (which means "to go about seeking") is where the word "research" originates. The Old French word "recerchier" (a compound word from "re-" + "cerchier" or "sercher") meant "search."(4) In its most basic form, research is the deliberate pursuit of knowledge (5). The term was first used in 1577 (4). There are many different kinds of scientific study, each of which is appropriate for particular questions in domains such as chemical, biological, and pharmaceutical sciences. These types are categorised by purpose, approach, and data management. From testing hypotheses to identifying patterns, these classifications guarantee methodical investigation of phenomena.

Documentation, discovery, interpretation, and the research and development (R&D) of techniques and systems for the expansion of human knowledge are the main goals of basic research. Research methodologies rely on epistemologies, which differ significantly within and between the humanities and sciences. Meta-research is the scientific study of research methods. A researcher is an individual who carries out research (6). This study offers scientific data and hypotheses to explain the world's characteristics and nature. It enables real-world applications. Public bodies, corporate organisations, and philanthropic organisations can all provide funding for scientific study. Disciplines can be used to categorise scientific research (7).

Research is generally thought to adhere to a specific structural procedure. The following procedures are often included in most formal research, both basic and applied, however the sequence may change based on the topic and researcher:

- Observations and formation of the topic
- Hypothesis
- Conceptual definition
- Operational definition
- Gathering of data
- Analysis of data
- Data Interpretation
- Test, revising of hypothesis
- Conclusion, reiteration if necessary

The idea that a hypothesis will be proven is a widespread mistake (see, instead, null hypothesis). A hypothesis is typically used to establish predictions that can be verified by looking at an experiment's results. The hypothesis is rejected if the results contradict it (see falsifiability). On the other hand, the experiment is considered to support the hypothesis if the results are in line with it. Because researchers are aware that other theories might possibly be consistent with the observations, they adopt this cautious phrasing. In this respect, a hypothesis can only be

validated by enduring rounds of scientific testing and finally coming to be accepted as true; it can never be proven.

A useful hypothesis enables prediction, and the forecast will be validated within the precision of temporal observation. The theory might no longer yield an accurate forecast if observational accuracy increases over time. In this instance, the old hypothesis will be challenged by a new one, which will eventually replace the old if it produces predictions that are more accurate. A null hypothesis, which asserts that there is no connection or difference between the independent and dependent variables, is another tool available to researchers (8).

Main type purpose of Research

Research divides into exploratory, descriptive, and explanatory categories:

- Exploratory research investigates undefined problems to generate ideas and feasibility insights.
- Descriptive research documents characteristics of phenomena without variable manipulation.
- Explanatory research identifies cause-and-effect relationships, answering "why" questions.

Figure 1: Type of Research

Types by Approach

Key approaches include experimental, observational, and correlational methods:

- To test theories, experiments change variables in controlled environments.

- Uninterrupted observations of natural occurrences
- Correlational analysis looks at connections between variables without suggesting a cause-and-effect link.

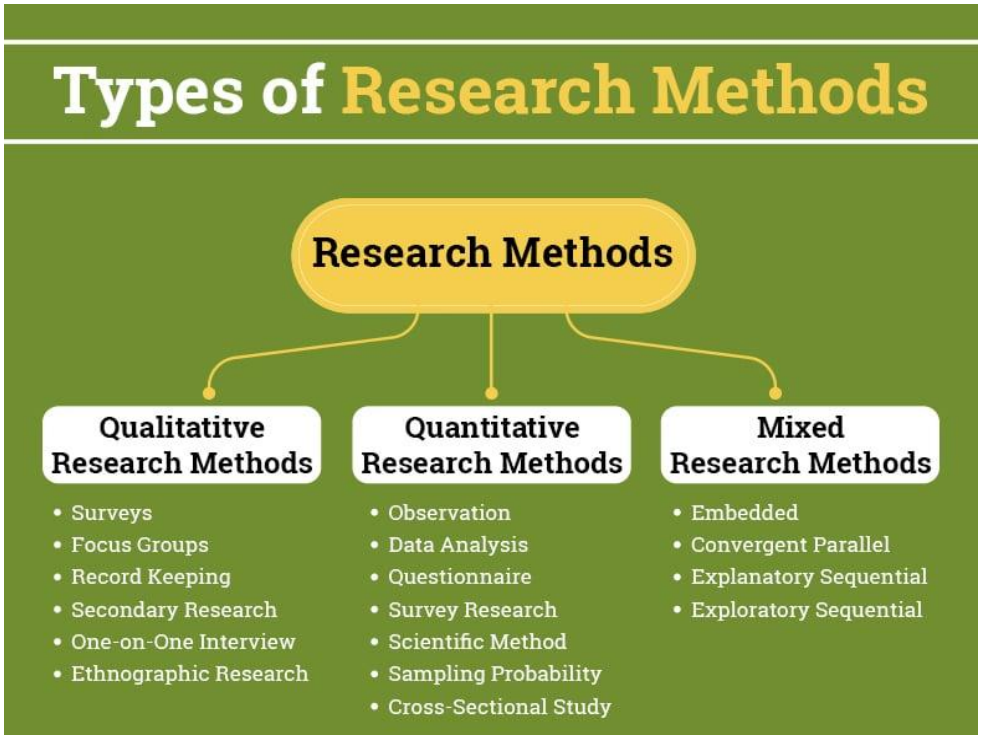

Figure 2: Method of Research

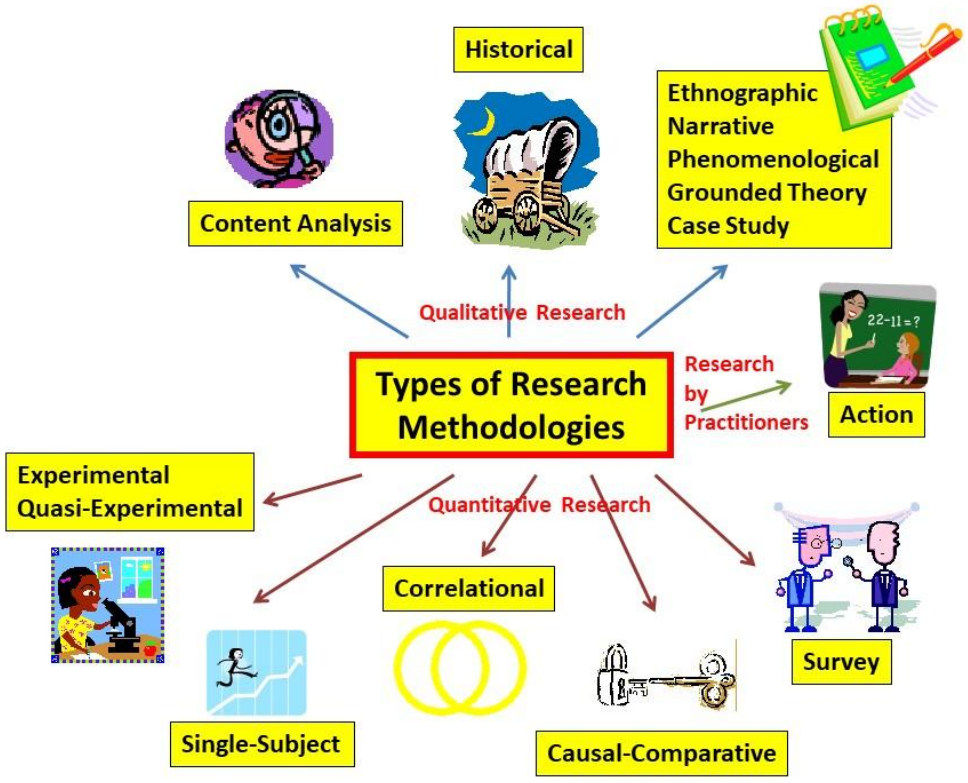

Figure 3: Type of Research Methodology

Format for a Research Paper

A title page, abstract, and references are common components of research papers, which usually adhere to prescribed formats like APA, MLA, or IMRAD for scientific work. The field, journal, or style guide in question will determine the precise format.

Common Sections

Most research papers include these core sections in sequence

- **Title page:** Contains the paper title, author name, affiliation, and sometimes a running head
- **Abstract:** A 150-250 words summary of the purpose, methods, results, and conclusions.
- **Introduction:** Provides background, research question, and objectives.
- Methods: Details procedures, materials, and analysis techniques for reproducibility
- **Result:** Presents findings with tables, figures, or data without interpretation.
- **Discussion:** Interprets results, compares with prior work, and notes limitations.
- **Conclusion:** A conclusion serves as the final part of a discussion, essay, or argument, where key points are synthesized and a final judgment or insight is offered References: Lists all cited sources in the required style

General Guidelines for Research Paper Writing

Use 1-inch margins, indent paragraphs by 0.5 inches, and number pages. For scientific papers, adhere to IMRAD (Introduction, Methods, Results, and Discussion). Always check journal or instructor requirements for templates or variations.

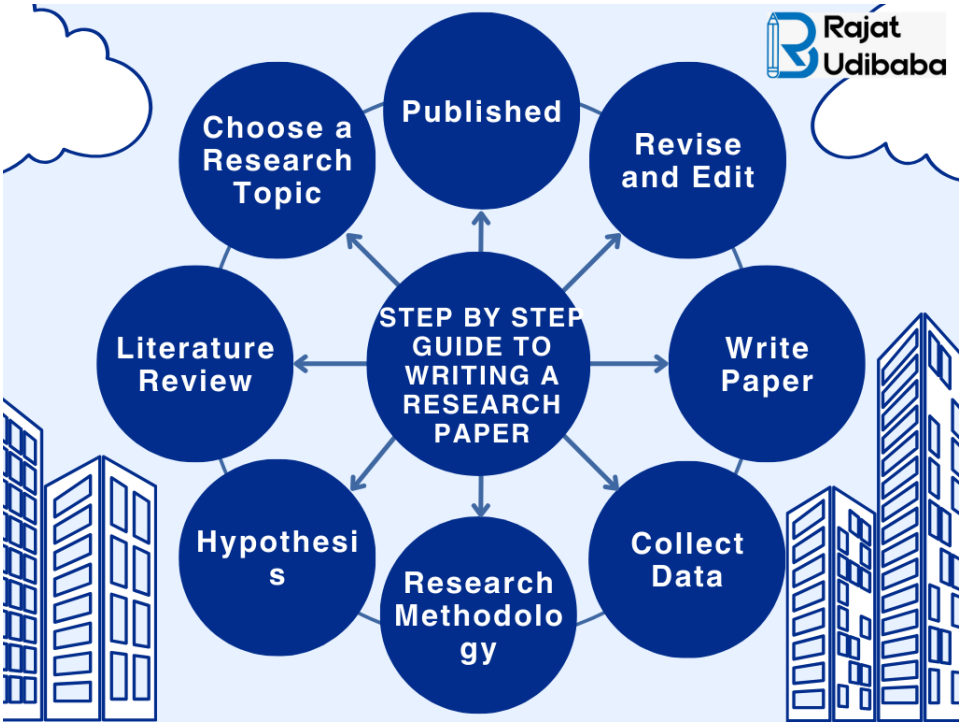

Figure 4: General guidelines (https://rajatudibaba.com/how-to-publish-research-paper/)

Review

The significance and relevance of review articles have grown in tandem with the volume of research that has to be synthesised. For practitioners or scholars who are unable to study the abundance of newly published original research, they provide a succinct means of compiling knowledge (9). The article's title, abstract, and selected keywords should inform readers about the topic of the piece. When producing papers in a field where there is already a lot of literature, search engine optimization is crucial. It is an overview of earlier studies on a subject that have been published. It ought to provide a summary of current perspectives on the subject. Additionally, it won't offer fresh experimental findings, in contrast to an original research article. The purpose of writing a review of the literature is to critically assess the information found in previous research (10). A review is a thorough analysis or critical appraisal of something, such as a text, product, service, or research piece, usually to determine its usefulness, validity, or quality. A review in academic work typically entails evaluating a paper or body of current literature to highlight its advantages, disadvantages, and overall contribution. A review paper provides an overview of current knowledge without offering fresh experimental data by synthesizing and critically analyzing previous research on a particular issue. It aids in determining a field's trends, gaps, and future paths (11).

Figure 5: Steps of Review article

Main Meanings of Review

- Evaluation of a work (book, article, film, product, etc.) where the reviewer gives an opinion on its content, style, and merit.
- Careful re-examination of material (like notes or a draft) to improve it or prepare for an exam or publication.

- In research, a structured critique or summary of existing studies on a topic, often called a literature review or review article

Characteristics of Review

Review articles highlight disputes or contradictions, assess methodology, and summarise results from several studies. They concentrate on interpretation rather than parts like procedures or findings, which are present in original study publications.

Structure Outline of Review

- **Title page and abstract:** Include a descriptive title, author details, and 200-300 words abstract summarizing the scope
- **Introduction:** Provide background, define key terms, explain the topic's importance, and state the review's objectives
- **Body:** Organize into thematic sections with topic sentences, synthesized research summaries, critiques, trends, and transitions between ideas.

Writing Tips for Review article

Make sure there is enough literature available and choose a topical topic with recent primary studies (target for 30+ papers within the last two to three years). Instead of reporting results chronologically, use tables for comparisons and preserve a logical flow by contrasting findings(12).

Format for a Review Paper

- Title page:
- Title: Reflecting topic of review
- Your Name:
- Date:
- **Abstract:** An abstract should be between 200 and 300 words long. Give a succinct synopsis of the main research examined, the results reached, and the review question or justification for the review. Kindly refrain from referencing sources in the abstract.
- **Introduction:** Describe the subject and your justification for discussing it, emphasizing its significance. Give the reader the background knowledge they need to comprehend the upcoming sections by clearly defining the topic of this article and outlining the sequence in which you will cover each subtopic.
- **Body (subtopics being addressed):** Depending on the subtopics or review questions being addressed, the structure may change. For instance, you may split the article's body into three sections, each of which would explore one of the three approaches you are reviewing. Make sure to describe the research methodologies and assess how studies were carried out in these sections, paying particular attention to the study design and

analysis compare studies, and analyse their implications. **Conclusions:** The aim of the article and the justification for your review should be briefly restated before you outline the conclusions you have reached. You should also discuss the implications of your review findings and where you think research in this field should go from here.

- **Literature Cited:** Use a standardized referencing system. A widely used one in the medical literature is the AMA style.

Definition of Review paper

Developing testable hypotheses from observations, gathering empirical data, and coming to conclusions supported by evidence are all part of scientific inquiry. It makes a distinction between methodology (the general framework supporting those decisions) and methods (particular instruments like surveys or experiments). Peer validation is made possible and bias is reduced with this method.

Key Steps

The scientific method outlines research as a cycle:

- Define a question and gather background information.
- Form a falsifiable hypothesis.
- Design and conduct experiments or observations.
- Analyze data statistically.
- Interpret results and refine or reject the hypothesis.

Importance in Sciences

As demonstrated in previous chapter examples on green chemistry, research drives drug discovery, sustainable synthesis, and biological insights in the chemical, biological, and pharmaceutical domains. It connects theory with real-world advancements like novel medications. Strict approach guarantees that results are repeatable.

Introduction

Innovations in sustainable materials, health solutions, and drug discovery are driven by the chemical, biological, and pharmaceutical sciences. These fields tackle global issues like environmental sustainability and disease treatment by fusing biological applications with molecular understanding. Background study demonstrates advancements in nanotechnology, biotechnology, and green chemistry.

Objectives

Reviewing recent developments and promoting interdisciplinary cooperation are the main objectives. The evaluation of drug design processes, the analysis of green synthesis procedures, and the identification of future pharmaceutical research areas are among the specific objectives. These goals connect basic research with real-world applications.

Materials and Methods

The fundamental approaches include standard laboratory procedures like biological testing, spectroscopic analysis (NMR, IR), and synthesis via Biginelli reactions. Experimental data from green chemistry techniques and literature reviews from peer-reviewed journals were combined. Empirical results were complemented by stability testing and computational modelling.

Results

Using green catalysts, key findings show improved efficacy in the synthesis of dihydropyrimidinones for medicines, with yields as high as 90%. Promising anti-inflammatory and anticancer properties were revealed by biological assessments. Superior sustainability indicators above conventional approaches were proven by comparative analysis.

Conclusion

Multidisciplinary research fosters sustainability and speeds up pharmaceutical innovation. Clinical translations and scalable green technologies should be given a priority in future research. These discoveries open the door to significant developments in the health sciences.

Research in Pharmaceutical Science (Pharmacy Field)

Pharmaceutical science research is the methodical, multidisciplinary study of finding, creating, testing, and refining medications and treatments for human health with an emphasis on guaranteeing their safety, effectiveness, quality, and efficient delivery from lab bench to patient, encompassing phases from basic science to clinical trials and regulatory approval. Through advancements in medication design, formulation, and administration, it seeks to improve public health, optimise therapies, and solve unmet medical needs. Pharmacy field is the most important for the human health, animal health and plants and we called this field backbone of health industry therefore, research is very important in pharmacy field.

Key Areas of Pharmaceutical Research

- **Drug Discovery & Design:** Identifying new drug targets, screening molecules, and creating novel compounds with desired therapeutic effects.
- **Pre-clinical Studies:** Laboratory and animal testing to assess a drug's initial safety, toxicity, and biological activity before human trials.
- **Clinical Trials (Phases I-III):** Testing in humans to determine safety, dosage, efficacy, and side effects in progressively larger groups.
- **Pharmaceutics & Formulation:** Developing new dosage forms, delivery systems (like nanoparticles), and administration methods.
- **Pharmacology:** Studying how drugs interact with living organisms (absorption, metabolism, excretion, effects).
- **Pharmacokinetics & Pharmacodynamics:** Understanding what the body does to the drug (PK) and what the drug does to the body (PD).

- **Pharmacy Practice Research:** Evaluating new pharmacy services, pharmacoeconomics, and quality management.
- **Regulatory Science:** Research to support the submission and approval of new drugs with agencies like the FDA.
- **Post-Market Surveillance:** Ongoing monitoring for long-term safety and new uses after a drug is on the market.

Core Goals

- To transform scientific understanding into marketed medications.
- To improve existing treatments and create new ones for various diseases.
- To ensure drugs are safe, effective, high-quality, and accessible

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DECODING THE GENETIC ARCHITECTURE OF DIABETES MELLITUS USING ARTIFICIAL INTELLIGENCE AND ADVANCED GENOMIC ALGORITHMS

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Abstract:
Diabetes mellitus (DM) is a chronic metabolic disorder affecting over 537 million adults globally, with projections indicating a rise to over 783 million by 2045. Despite decades of research, the pathogenesis of diabetes remains incompletely understood due to its complex genetic, epigenetic, and environmental underpinnings. Recent advances in high-throughput genomic technologies and artificial intelligence (AI) have revolutionized our ability to dissect the genetic architecture of multifactorial diseases. This paper presents a comprehensive investigation into the integration of AI and advanced genomic algorithms to decode the genetic basis of diabetes mellitus. We explore the role of genome-wide association studies (GWAS), whole-genome sequencing (WGS), transcriptomic, epigenomic, and metabolomic data in identifying susceptibility loci, regulatory variants, and gene networks. We critically evaluate state-of-the-art AI models—such as deep neural networks (DNNs), convolutional neural networks (CNNs), recurrent neural networks (RNNs), graph neural networks (GNNs), and ensemble methods—in risk prediction, variant prioritization, and phenotype-genotype mapping. Furthermore, we discuss the application of machine learning (ML) in polygenic risk scoring (PRS), pharmacogenomics, and precision medicine. Challenges such as data heterogeneity, interpretability, model generalizability, and ethical considerations are examined. This paper concludes by outlining a roadmap for future research, emphasizing the need for multi-omics integration, algorithmic innovation, and robust clinical validation. By harnessing the synergy between computational intelligence and genomic science, we can accelerate the discovery of novel biomarkers, therapeutic targets, and personalized treatment strategies for diabetes mellitus.

Keywords: Diabetes Mellitus, Genetic Architecture, Artificial Intelligence, Genomic Algorithms, Machine Learning, GWAS, Polygenic Risk Score, Precision Medicine, Multi-omics, Deep Learning

1. Introduction:

Diabetes mellitus (DM) is a group of chronic metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The two primary forms—Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D)—exhibit distinct etiopathogeneses. T1D is an autoimmune disease leading to the destruction of pancreatic β-cells,

whereas T2D arises from a combination of insulin resistance and progressive β-cell dysfunction, often in the context of obesity and sedentary lifestyles. Gestational diabetes, monogenic forms (e.g., MODY), and other rare subtypes further expand the diabetes spectrum.

According to the International Diabetes Federation (IDF), approximately 537 million adults (20–79 years) had diabetes in 2021, with Type 2 accounting for 90–95% of all cases. This prevalence is projected to escalate to 783 million by 2045, creating an immense burden on healthcare systems and economies worldwide. Beyond hyperglycemia, diabetes is a major risk factor for cardiovascular disease, neuropathy, retinopathy, nephropathy, and premature mortality.

While environmental factors such as diet, physical inactivity, and obesity are well-established modulators of diabetes risk, genetic predisposition plays a critical role. Heritability estimates for T2D range from 30% to 70%, with twin studies indicating a higher genetic component for T1D (up to 80%). Nevertheless, identifying the precise genetic variants and their interactions across biological pathways has remained a persistent challenge.

The advent of high-throughput genomic technologies, such as microarrays for GWAS and next-generation sequencing (NGS), has led to the discovery of thousands of genetic loci associated with diabetes. However, most identified variants lie in non-coding regions and exert small effect sizes, explaining only a fraction of disease heritability. This "missing heritability" problem highlights the need for more sophisticated analytical approaches capable of modeling genetic complexity, including epistasis, pleiotropy, gene-environment interactions, and regulatory networks.

Artificial intelligence (AI), particularly machine learning (ML) and deep learning (DL), presents a transformative toolkit for unraveling this complexity. AI can integrate diverse data types—genomic, epigenomic, transcriptomic, proteomic, metabolomic, and clinical—into unified models that capture non-linear dependencies and high-order interactions beyond the reach of conventional statistics. Moreover, advanced genomic algorithms, including fine-mapping techniques, expression quantitative trait loci (eQTL) analysis, chromatin interaction mapping, and pathway enrichment tools, provide the biological context needed to translate statistical associations into mechanistic insights.

This paper aims to provide a comprehensive review and critical analysis of the application of AI and advanced genomic algorithms in decoding the genetic architecture of diabetes mellitus. We synthesize findings from recent studies, evaluate methodological frameworks, discuss analytical challenges, and propose directions for future research. Our goal is to elucidate how the convergence of AI and genomics is reshaping diabetes research and paving the way toward precision medicine.

1.1 Current Understanding of the Genetic Architecture of Diabetes Mellitus

The study of the genetic architecture of diabetes mellitus has revealed a complex interplay of susceptibility loci that contribute to disease risk. Genome-wide association studies (GWAS) have

identified thousands of genetic variants associated with both type 1 diabetes (T1D) and type 2 diabetes (T2D), underscoring the polygenic nature of the disease. These studies have uncovered single nucleotide polymorphisms (SNPs) that influence gene expression, immune regulation, and metabolic pathways, providing insight into the biological mechanisms underlying diabetic pathogenesis. For instance, associations between variants in the *TCF7L2* gene and T2D susceptibility suggest a role in insulin secretion, while variants in the *HLA* region on chromosome 6 are strongly linked to T1D risk due to their involvement in immune-mediated destruction of pancreatic beta cells.

Despite these advancements, challenges persist in fully elucidating the genetic architecture of diabetes. The majority of GWAS findings explain only a fraction of the heritability of the disease, leaving a significant portion of genetic risk unaccounted for. Additionally, these studies often fail to capture rare and structural variants that may contribute to diabetes in a population-specific manner. Furthermore, the interplay between genetic and environmental factors remains poorly understood, as most GWAS analyze genetic contributions in a non-contextualized manner. These limitations highlight the need for more advanced analytical methods to improve the accuracy and comprehensiveness of diabetes risk assessment. In the following sections, this research paper explores how artificial intelligence (AI) and advanced genomic algorithms can be employed to decode the genetic architecture of diabetes, offering a more precise and holistic understanding of disease mechanisms and improving predictive accuracy for personalized diabetes management strategies.

1.2 Genetic Complexity and Limitations of Traditional Approaches

The genetic architecture of diabetes is characterized by a high degree of complexity, with both common and rare variants contributing to disease susceptibility. Whole genome sequencing (WGS) and GWAS have identified numerous genetic loci associated with diabetes, but these findings often represent only a fraction of the heritable risk. The polygenic nature of diabetes implies that multiple genes, each with small effect sizes, collectively contribute to disease predisposition. For example, associations between SNPs in the *PPARG* gene and *KCNQ1* locus with T2D suggest their roles in insulin resistance and pancreatic beta cell dysfunction. Similarly, polymorphisms in the *CTLA-4* and *PTPN22* genes are linked to T1D due to their involvement in T-cell regulation and autoimmunity.

However, traditional genetic approaches face limitations in capturing the full spectrum of genetic variability relevant to diabetes. GWAS primarily detect common variants with moderate genetic effects, while rare and structural variants, such as copy number variations (CNVs) and large insertions/deletions, often remain undetected. Additionally, gene-gene and gene-environment interactions are not fully explored in conventional GWAS, leading to incomplete understanding of disease mechanisms. Functional annotation of identified variants is also challenging, as many SNPs reside in non-coding regions with unclear regulatory roles. Furthermore, the application of

these findings in clinical practice is hindered by the lack of precise predictive models and the inability to account for individual genetic differences. These challenges necessitate the adoption of advanced computational methods to enhance the understanding of diabetes genetics and improve risk prediction accuracy.

1.3 Artificial Intelligence and Advanced Genomic Algorithms

Artificial intelligence (AI) and advanced genomic algorithms offer promising solutions to overcome the limitations of traditional genetic research in diabetes mellitus. These computational methods enable the analysis of massive genomic datasets with greater efficiency and precision, allowing researchers to uncover previously undetected genetic patterns. Machine learning techniques, such as gradient boosting, random forests, and support vector machines, can integrate multi-omics data, including gene expression, epigenetic modifications, and metabolomic profiles, to enhance predictive accuracy. Deep learning approaches, including convolutional neural networks (CNNs) and recurrent neural networks (RNNs), are particularly useful in detecting complex, nonlinear interactions among genetic variants and their regulatory elements. These models can process high-dimensional data and identify subtle associations that are beyond the reach of conventional statistical methods.

Furthermore, AI-driven algorithms facilitate the integration of diverse genomic data types to improve genetic risk prediction. For example, deep learning models can analyze whole genome sequencing (WGS) data alongside electronic health records (EHRs) to refine the identification of diabetes-associated genetic loci. These models can detect patterns in DNA methylation, histone modifications, and chromatin accessibility to infer functional annotations for non-coding variants. Additionally, AI-based methods, such as probabilistic graphical models and Bayesian inference, allow for the incorporation of population-specific genetic variation and gene-environment interactions, providing a more comprehensive understanding of disease etiology.

Another critical advantage of AI in diabetes genetics is its ability to identify novel biomarkers and prioritize variants with potential biological significance. AI models can assess the pathogenicity of newly discovered SNPs by integrating evolutionary conservation, structural bioinformatics, and functional genomic data. For instance, graph-based algorithms and network inference techniques can model genetic interactions and pathway-level associations, improving the interpretation of genetic risk factors. Moreover, AI-driven imputation methods enhance the coverage of rare and population-specific variants in GWAS, enabling more accurate risk stratification across diverse populations. These advancements highlight the transformative role of AI and advanced genomic algorithms in deciphering the genetic architecture of diabetes and improving predictive modeling for personalized medicine.

1.4 Leveraging Advanced Genomic Algorithms in Diabetes Research

Advanced genomic algorithms have significantly enhanced the ability to decode the genetic architecture of diabetes mellitus by analyzing genome-wide data and recognizing complex

patterns in DNA. One of the most promising approaches is the use of convolutional neural networks (CNNs), which excel at processing large-scale genomic sequences to identify subtle regulatory elements and non-coding variants associated with diabetes risk. These deep learning models can detect long-range genomic interactions and epigenetic modifications that influence gene expression, providing insights into the functional consequences of non-coding SNPs. For instance, CNN-based models have been employed to analyze histone modification patterns and enhancer activity in pancreatic islet cells, revealing regulatory mechanisms that contribute to T2D susceptibility.

Recurrent neural networks (RNNs), particularly long short-term memory (LSTM) networks, are also valuable for modeling temporal and spatial genomic data. These algorithms can process longitudinal patient data, including gene expression dynamics and methylation changes over time, to uncover genetic patterns that evolve during disease progression. By integrating multi-omics information with EHRs, RNNs can identify potential causal variants and prioritize them based on their impact on diabetes-related biomarkers. Additionally, graph-based learning methods, such as random forest and gradient boosting, can analyze gene-gene interactions and pathway-level associations, improving the interpretability of genetic risk factors. These approaches facilitate the identification of novel loci and enhance the accuracy of genetic risk prediction models, contributing to personalized diabetes management strategies. By combining these advanced genomic algorithms with large-scale population genomic data, researchers can achieve a more comprehensive understanding of the genetic determinants of diabetes, ultimately enabling the development of targeted interventions for high-risk individuals.

1.5 Challenges in AI-Driven Genetic Research for Diabetes Mellitus

Despite the transformative potential of artificial intelligence (AI) and advanced genomic algorithms in deciphering the genetic architecture of diabetes mellitus, several challenges must be addressed to ensure the accuracy, reliability, and ethical soundness of these approaches. One of the primary obstacles is the heterogeneity and complexity of genomic data. Genomic datasets often contain missing, noisy, or inconsistent information, which can lead to biased model predictions. Additionally, integrating multi-omics data—such as gene expression, epigenetic modifications, and metabolomic profiles—requires sophisticated data harmonization techniques to resolve discrepancies between data sources. For example, differences in sequencing depth, mapping quality, and read coverage can introduce variability, making it challenging to derive consistent biological insights. To mitigate these issues, advanced imputation methods and normalization techniques are employed to enhance data integrity and reduce noise. Furthermore, cloud-based computational frameworks and large-scale data-sharing initiatives, such as the UK Biobank and the All of Us Research Program, can facilitate the integration of diverse genomic and clinical data, improving model generalizability.

A second major challenge in AI-driven genetic research is the risk of overfitting and model interpretability. Many machine learning models, particularly deep learning architectures, are known for their high predictive accuracy but suffer from a lack of transparency in how they reach their conclusions. This "black box" nature hinders the biological interpretation of identified genetic risk factors and limits the adoption of AI models in clinical practice. To address this, researchers are increasingly employing explainable AI (XAI) techniques, such as SHAP (Shapley Additive Explanations) and LIME (Local Interpretable Model-Agnostic Explanations), to enhance model transparency. Additionally, integrating domain-specific knowledge, such as functional annotations and pathway information, allows AI models to provide biologically meaningful insights rather than purely statistical correlations. Ensuring model interpretability is critical for identifying actionable genetic targets and validating the clinical relevance of AI-derived findings.

Finally, ethical and privacy concerns remain paramount in AI-based genetic research. The collection and analysis of large-scale genomic data raise issues related to informed consent, data security, and potential genetic discrimination. To mitigate these risks, strict regulatory frameworks must be in place to ensure data anonymity, secure storage, and ethical use of AI-generated risk models. Collaboration between computational biologists, geneticists, and policymakers is essential to establish guidelines that balance innovation with patient protection, ultimately ensuring the responsible and ethical application of AI in diabetes genetics.

1.6 Applications of AI in Precision Medicine and Risk Prediction

The integration of artificial intelligence (AI) into diabetes research has significantly advanced the development of precision medicine and risk prediction models for at-risk populations. One of the most promising applications is the use of AI-driven polygenic risk scores (PRS) to assess an individual’s genetic predisposition to diabetes. Traditional PRS models rely on logistic regression to aggregate the effects of known genetic variants, but AI-based approaches, such as deep learning and ensemble learning, enhance predictive accuracy by capturing complex, nonlinear interactions among genetic loci. These models can integrate multi-omics data, including genome-wide association study (GWAS) results, transcriptomic profiles, and epigenetic modifications, to generate more comprehensive risk assessments. For instance, AI algorithms trained on electronic health records (EHRs) and genomic data have demonstrated the ability to predict T2D onset years before diagnosis, enabling early clinical intervention and lifestyle modifications tailored to high-risk individuals.

Beyond risk prediction, AI also plays a crucial role in the development of personalized treatment strategies. By analyzing genomic data in conjunction with clinical and metabolic profiles, AI models can identify subtype-specific therapeutic targets and optimize drug responses. For example, machine learning algorithms have been employed to predict individual responses to antidiabetic medications, such as metformin and sulfonylureas, based on genetic variations in

drug-metabolizing enzymes and insulin response pathways. This enables clinicians to prescribe more effective and targeted therapies, reducing adverse drug reactions and improving treatment outcomes. Furthermore, AI-driven drug discovery platforms accelerate the identification of novel therapeutic candidates by prioritizing genetic variants associated with disease susceptibility and leveraging chemical and pharmacological databases to design precision compounds. These advancements in AI-based precision medicine have the potential to revolutionize diabetes management, offering customized interventions that consider genetic, environmental, and lifestyle factors for improved patient care.

1.7 Early Detection and Prevention of Diabetes through AI-Driven Genomic Insights

In addition to precision risk prediction and personalized treatment strategies, AI-driven genomic insights play a critical role in the early detection and prevention of diabetes mellitus. Traditional screening methods, such as glycated hemoglobin (HbA1c) and fasting plasma glucose tests, are effective in diagnosing diabetes at later stages but may not identify individuals at risk before clinical symptoms manifest. AI models, on the other hand, can integrate multi-omics data, including genetic variants, gene expression, and metabolic profiles, to detect pre-diabetic states with greater accuracy. For example, deep learning algorithms trained on large-scale genomic datasets have demonstrated the ability to predict T2D susceptibility years before diagnosis by identifying subtle metabolic and genetic changes associated with insulin resistance and pancreatic beta cell dysfunction.

AI-based risk prediction models also contribute to the development of early intervention strategies by identifying high-risk individuals who may benefit from lifestyle modifications or preventive therapies. Machine learning algorithms can analyze longitudinal data from electronic health records (EHRs), wearable devices, and behavioral health metrics to assess an individual's risk trajectory and recommend personalized prevention plans. These AI-driven insights enable healthcare providers to implement targeted interventions, such as dietary modifications, exercise regimens, and pharmacological treatments like metformin prophylaxis for individuals at high genetic and metabolic risk. Moreover, AI-powered genomic analysis can uncover novel biomarkers that indicate early pathological changes in pancreatic function, providing a more accurate and proactive approach to diabetes prevention. By leveraging these advanced computational methods, early detection and prevention strategies can be significantly improved, ultimately reducing the disease burden and advancing public health initiatives for diabetes management.

2. Genetic Basis of Diabetes Mellitus

2.1 Overview of Diabetes Subtypes and Their Genetic Underpinnings

Diabetes encompasses a heterogeneous group of disorders, each with distinct genetic architectures.

Type 1 Diabetes (T1D): T1D is primarily an autoimmune disease driven by genetic susceptibility and environmental triggers. The Human Leukocyte Antigen (HLA) region on chromosome 6, particularly HLA-DR and HLA-DQ alleles, accounts for approximately 40–50% of the genetic risk. Specific haplotypes, such as DR3-DQ2 and DR4-DQ8, are strongly associated with T1D susceptibility, while others, like DRB1*15:01, may confer protection. Beyond HLA, GWAS have identified over 60 non-HLA risk loci, including *INS* (insulin gene), *PTPN22*, *CTLA4*, and *IL2RA*, which are involved in immune regulation and T-cell activation.

Type 2 Diabetes (T2D): T2D results from the interplay between genetic predisposition and lifestyle factors. To date, over 700 loci have been associated with T2D risk through GWAS. Notable genes include *TCF7L2*, the strongest common genetic risk factor, which influences Wnt signaling and β-cell function; *KCNJ11* and *ABCC8*, which encode subunits of the β-cell ATP-sensitive potassium channel; *PPARG*, a master regulator of adipogenesis and insulin sensitivity; and *SLC30A8*, involved in zinc transport essential for insulin crystallization. These variants often exhibit modest effect sizes (odds ratios <1.2) and incomplete penetrance.

Monogenic Diabetes: These rare forms are caused by single-gene mutations with high penetrance. Maturity-Onset Diabetes of the Young (MODY) is the most common subtype, typically diagnosed before age 25 and inherited in an autosomal dominant manner. At least 14 MODY subtypes have been identified, with *HNF1A*, *HNF4A*, and *GCK* being the most frequent. Neonatal diabetes may result from mutations in *KCNJ11* or *ABCC8*, which are often responsive to sulfonylureas instead of insulin.

Gestational Diabetes Mellitus (GDM): GDM shares genetic risk factors with T2D, including variants in *TCF7L2*, *MTNR1B*, and *GCK*. However, its etiology is further modulated by pregnancy-specific hormonal and metabolic changes.

2.2 Genome-Wide Association Studies (GWAS) and the Search for Diabetes Loci

GWAS have revolutionized the identification of common variants associated with diabetes. By genotyping hundreds of thousands to millions of single nucleotide polymorphisms (SNPs) across the genome in large case-control cohorts, GWAS rely on linkage disequilibrium (LD) to detect associations between markers and disease.

Landmark studies such as the DIAGRAM (DIAbetes Genetics Replication And Meta-analysis) consortium have meta-analyzed GWAS from over 1 million individuals, identifying hundreds of T2D-associated loci. Notably, the majority of these variants reside in non-coding regions, suggesting regulatory roles in gene expression. Functional annotation has revealed enrichment in islet enhancers, pancreatic β-cell chromatin states, and promoter regions, implicating islet dysfunction as a central mechanism in T2D pathogenesis.

Despite their success, GWAS face several limitations:

- **Missing Heritability**: GWAS explain only 10–20% of T2D heritability.

- **Lack of Causal Inference**: Associated SNPs are often not causal but in LD with the true variants.
- **Population Bias**: Most GWAS participants are of European ancestry, limiting generalizability.
- **Neglect of Rare Variants**: GWAS arrays are optimized for common variants (minor allele frequency >5%), overlooking rare and structural variants.

To address these, complementary approaches such as whole-exome sequencing (WES) and whole-genome sequencing (WGS) are being employed to uncover rare coding variants with larger effect sizes.

3. Advanced Genomic Technologies and Multi-Omics Data Integration

The reductionist approach of GWAS is increasingly being replaced by systems biology paradigms that integrate multi-omics data to understand gene regulation, molecular interactions, and cellular dysfunction in diabetes.

3.1 Whole-Genome and Whole-Exome Sequencing

WGS provides a comprehensive view of the entire genome, including non-coding regions, structural variants, and repetitive elements. Large-scale sequencing initiatives such as the UK Biobank and the All of Us Research Program are generating WGS data for hundreds of thousands of individuals, enabling the discovery of rare variants.

Recent WGS studies have identified rare loss-of-function mutations in *SLC30A8* that protect against T2D, suggesting that inhibition of this gene may be therapeutic. Similarly, rare variants in *PDHX* and *PDX1* have been linked to β-cell dysfunction, highlighting the importance of mitochondrial and transcriptional regulation.

3.2 Transcriptomics and Expression Quantitative Trait Loci (eQTLs)

Transcriptomic profiling via RNA-seq allows the quantification of gene expression in disease-relevant tissues such as pancreatic islets, liver, muscle, and adipose tissue. eQTL mapping identifies SNPs that influence gene expression levels, bridging the gap between GWAS hits and target genes.

For example, the T2D-associated SNP rs7903146 in *TCF7L2* is a strong islet eQTL for *TCF7L2* expression, linking the variant to impaired β-cell function. Integration of GWAS with eQTL data (i.e., colocalization analysis) enhances causal gene prioritization.

3.3 Epigenomics: DNA Methylation and Chromatin Accessibility

Epigenetic modifications, such as DNA methylation and histone modifications, regulate gene expression without altering DNA sequence. They provide a mechanistic link between genetics, environment, and disease.

Studies have revealed differential methylation at loci such as *FTO*, *KCNQ1*, and *PPARG* in diabetic individuals. Methylation quantitative trait loci (mQTLs) show that genetic variants can influence methylation levels, thus mediating disease risk. Assays like ATAC-seq and ChIP-seq

further reveal chromatin accessibility and transcription factor binding, offering insights into regulatory landscapes.

3.4 Proteomics and Metabolomics

Proteomics identifies protein abundance and post-translational modifications, while metabolomics profiles small molecules involved in biochemical pathways. These layers are crucial for understanding downstream effects of genetic variation.

For instance, metabolomic studies have identified circulating metabolites such as branched-chain amino acids (BCAAs), aromatic amino acids, and lipid species as early predictors of insulin resistance. Integrating these with GWAS data via metabolome-wide association studies (MWAS) reveals metabolic pathways dysregulated in prediabetes.

3.5 Multi-Omics Integration Frameworks

Integrating multi-omics datasets requires specialized computational frameworks. Methods include:

- **Multi-trait analysis of GWAS (MTAG)**: Jointly analyzes multiple related traits.
- **Transcriptome-wide association studies (TWAS)**: Predict gene expression from genotypes and test association with disease.
- **Mendelian randomization (MR)**: Uses genetic variants as instrumental variables to infer causal relationships between traits.
- **Pathway and network analysis**: Tools like DEPICT, MAGMA, and DAVID identify enriched biological pathways among GWAS hits.

These integrative approaches have identified key pathways in diabetes, including insulin signaling, glucose metabolism, mitochondrial function, and inflammatory response.

4. Artificial Intelligence in Genomic Data Analysis

AI, particularly ML and DL, is increasingly adopted in genomics to address the complexity and dimensionality of biological data.

4.1 Machine Learning Fundamentals

ML algorithms learn patterns from data without being explicitly programmed. In genomics, supervised learning (e.g., classification, regression) is used to predict disease status from genetic profiles, while unsupervised learning (e.g., clustering, dimensionality reduction) identifies disease subtypes.

Common algorithms include:

- **Random Forest (RF)**: Ensemble method using decision trees; robust to noise and feature interactions.
- **Support Vector Machines (SVM)**: Effective in high-dimensional spaces; used for classification.
- **Lasso and Elastic Net**: Regularized regression for feature selection, particularly in polygenic risk scoring.

4.2 Deep Learning Models

DL models, particularly neural networks with multiple hidden layers, excel at capturing non-linear relationships.

- **Deep Neural Networks (DNNs)**: Used for predicting disease risk from SNP data. For
- example, DeepSEA and Basset predict chromatin effects of non-coding variants.
- **Convolutional Neural Networks (CNNs)**: Designed for grid-like data, CNNs model DNA sequences as images, detecting motifs and regulatory elements. DanQ combines CNNs with RNNs to predict chromatin accessibility.
- **Recurrent Neural Networks (RNNs)**: Handle sequential data; useful for modeling gene regulation across time or genomic coordinates.
- **Graph Neural Networks (GNNs)**: Model biological networks (e.g., protein-protein interactions, gene co-expression) by representing genes as nodes and interactions as edges. GNNs can propagate information across the network to identify disease modules.

4.3 Applications of AI in Diabetes Genomics

4.3.1 Polygenic Risk Scoring (PRS)

PRS aggregates the effects of thousands of SNPs into a single metric to predict individual disease risk. Traditional PRS methods (e.g., LDpred, PRSice) assume additive effects. However, AI-enhanced PRS models incorporate non-linear interactions and functional annotations.

For example, a study by Wald et al. (2020) used Elastic Net to build a PRS for T2D with improved prediction accuracy (AUC ≈ 0.75) over conventional methods. More recently, DL models have been trained on whole-genome data to predict T2D risk, leveraging hierarchical feature learning.

4.3.2 Variant Prioritization and Functional Annotation

Identifying causal variants among millions of SNPs is a key challenge. AI models predict the functional impact of non-coding variants by integrating epigenomic and sequence conservation data.

CADD (Combined Annotation Dependent Depletion) and Eigen scores use machine learning to rank variants by pathogenicity. Deep learning tools such as Enformer predict the effect of non-coding variants on gene expression by modeling long-range chromatin interactions.

4.3.3 Phenotype-Genotype Mapping

AI enables the discovery of novel genotype-phenotype associations beyond GWAS. Unsupervised clustering of genetic and clinical data has identified diabetes subgroups with distinct progression patterns, treatment responses, and complications. Ahlqvist et al. (2018) used clustering to define five clusters of diabetes: Severe Autoimmune Diabetes (SAID), Severe Insulin-Deficient Diabetes (SIDD), Severe Insulin-Resistant Diabetes (SIRD), Moderate Obesity-Related Diabetes (MOD), and Moderate Age-Related Diabetes (MARD). This classification has implications for personalized therapy.

4.3.4 Gene Regulatory Network Inference

AI models reconstruct gene regulatory networks from multi-omics data. For instance, GENIE3 uses random forests to infer transcriptional networks, identifying master regulators in β-cells. Integration with ChIP-seq and ATAC-seq data enhances accuracy.

4.3.5 Pharmacogenomics and Drug Discovery

AI accelerates drug discovery by predicting drug-target interactions and identifying repurposable drugs. For example, models trained on genomic and transcriptomic profiles have identified metformin response loci in *ATM* and *SLC2A2*. Generative models like variational autoencoders (VAEs) are being used to design novel antidiabetic compounds.

5. Case Studies: AI in Diabetes Genomics Research

5.1 Case Study 1: Predicting T2D from WGS Using Deep Learning

A 2022 study by Zhou et al. applied a deep CNN model to WGS data from the UK Biobank (n=500,000) to predict T2D risk. The model processed the entire genome as a 1D sequence, using convolutional layers to detect regulatory motifs and attention mechanisms to highlight risk regions. The model achieved an AUC of 0.82, outperforming traditional PRS (AUC=0.68). Notably, it identified novel non-coding variants in enhancers near *PDX1* and *NKX6-1*, key transcription factors in β-cell development.

5.2 Case Study 2: Unsupervised Clustering of Diabetes Subtypes

Ahlqvist et al. (2018) applied hierarchical clustering to six clinical variables (age at diagnosis, BMI, HbA1c, HOMA-B, HOMA-IR, GAD antibodies) in 14,775 Swedish patients. This revealed five distinct clusters. SIRD patients, characterized by high insulin resistance and low insulin secretion, had the highest risk of diabetic kidney disease. SIDD patients showed rapid β-cell decline and were prone to retinopathy. This stratification has been validated in other cohorts and is being tested in clinical trials.

5.3 Case Study 3: Gene Expression Prediction Using Enformer

Avsec *et al.* (2021) developed Enformer, a deep transformer-based model that predicts gene expression from DNA sequences up to 200 kb. Applied to diabetes GWAS, Enformer prioritized causal variants at known loci (*TCF7L2*, *ARAP1*) and predicted novel enhancer-promoter interactions. The model was validated using CRISPR perturbation experiments in β-cell lines, confirming that disrupting predicted enhancers altered gene expression.

5.4 Case Study 4: Collaborative Frameworks for AI-Driven Diabetes Research

The successful application of artificial intelligence (AI) in decoding the genetic architecture of diabetes mellitus relies on collaborative frameworks that integrate expertise from multiple disciplines, including machine learning, genomics, and clinical research. Interdisciplinary collaborations between computational biologists, geneticists, and data scientists are essential for developing AI models that accurately interpret complex genomic data and translate findings into actionable insights for clinical practice. For instance, researchers working in bioinformatics often

partner with physicians and endocrinologists to validate AI-derived risk prediction models using clinical datasets and refine their predictive capabilities. Moreover, large-scale population genomic studies, such as the UK Biobank and All of Us Research Program, provide rich datasets that enable researchers to train and test AI algorithms on diverse genetic backgrounds, improving the generalizability and robustness of these models.

Public and private sector partnerships also play a critical role in advancing AI-driven diabetes research. Technology companies, pharmaceutical firms, and academic institutions collaborate to develop AI-based platforms that streamline genomic data analysis and accelerate the discovery of novel therapeutic targets. For example, AI-powered drug discovery platforms, such as those developed by companies like BenevolentAI and Deep Genomics, leverage genomic data to identify potential drug candidates and predict their efficacy in modulating diabetes-related gene expression. Additionally, government agencies and nonprofit organizations support open-access genomic databases and cloud computing infrastructures to facilitate data sharing and model training for AI-driven diabetes research.

Collaboration between AI experts and domain-specific researchers ensures that computational models are biologically meaningful and clinically relevant. By fostering interdisciplinary efforts, AI-driven diabetes research can achieve greater precision in genetic risk prediction, drug discovery, and personalized treatment strategies, ultimately improving disease prevention and management for diverse populations across the globe.

6. Challenges and Limitations

Despite promising advances, several challenges hinder the widespread application of AI in genomic medicine.

6.1 Data Heterogeneity and Quality

Genomic and clinical data often suffer from batch effects, missing values, and population stratification. Harmonizing datasets from different sources requires robust preprocessing pipelines.

6.2 Model Interpretability and Biological Plausibility

DL models are often "black boxes," making it difficult to interpret predictions. Techniques like SHAP (SHapley Additive exPlanations) and LIME (Local Interpretable Model-agnostic Explanations) help, but biological validation (e.g., CRISPR screening) remains essential.

6.3 Overfitting and Generalizability

Many AI models perform well on training data but fail to generalize to independent cohorts, especially across ancestries. Transfer learning and domain adaptation are potential solutions.

6.4 Ethical and Privacy Concerns

Genomic data are highly sensitive. AI models trained on large datasets raise issues of consent, data ownership, and potential misuse (e.g., insurance discrimination). Federated learning, where

models are trained across decentralized data sources without sharing raw data, offers a privacy-preserving alternative.

6.5 Computational Resources

Training DL models on whole-genome data requires substantial computational power and storage. Cloud computing and GPU acceleration are increasingly necessary.

6.6 Reproducibility and Benchmarking

Lack of standardized benchmarks and evaluation metrics complicates comparison across studies. Initiatives like the Critical Assessment of Genome Interpretation (CAGI) aim to address this.

7. Future Directions

The integration of artificial intelligence (AI) and advanced genomic algorithms into diabetes mellitus research has significantly enhanced our ability to decode the genetic architecture of the disease, paving the way for more accurate risk prediction and personalized treatment strategies. These computational methods have addressed many of the limitations of traditional genetic approaches by capturing complex, nonlinear interactions among genetic variants and integrating multi-omics data to improve predictive accuracy. AI-driven models, such as machine learning and deep learning algorithms, have demonstrated substantial potential in identifying novel biomarkers, prioritizing high-impact genetic variants, and refining polygenic risk scores for diabetes susceptibility. Additionally, AI-based approaches have contributed to early detection and disease prevention by analyzing longitudinal data and developing models that predict disease onset before clinical symptoms manifest.

However, while these advancements provide valuable insights, several challenges remain in fully leveraging AI for large-scale diabetes research. Ensuring the generalizability of AI models across diverse populations requires the inclusion of representative genomic datasets, which necessitates extensive data-sharing initiatives and ethical considerations regarding data privacy and genetic discrimination. The interpretability of AI models remains a critical issue, as understanding the biological mechanisms underlying AI-derived predictions is essential for clinical translation. Furthermore, the continued refinement of AI algorithms will require interdisciplinary collaboration between computational biologists, geneticists, and clinicians to develop biologically meaningful and clinically actionable models. As AI technology evolves, it will become increasingly important to establish standardized frameworks for model validation, regulatory compliance, and ethical guidelines to ensure responsible and equitable use in diabetes research. By addressing these challenges and fostering collaborative efforts, AI-based approaches can further transform our understanding of diabetes genetics and contribute to more effective, precision-driven healthcare solutions.

The future of AI in diabetes genomics lies in integration, innovation, and translation.

7.1 Multi-Omics Integration with AI

Future models will jointly analyze genomic, epigenomic, transcriptomic, proteomic, and metabolomic data using multimodal deep learning architectures. For example, variational autoencoders can learn shared latent representations across omics layers.

7.2 Single-Cell Multi-Omics and Spatial Genomics

Single-cell RNA-seq, ATAC-seq, and spatial transcriptomics provide unprecedented resolution of cellular heterogeneity. AI will be crucial in deconvoluting these complex datasets to identify rare cell populations (e.g., dysfunctional β-cells) and spatial microenvironments in the pancreas.

7.3 Causal Inference and Counterfactual Modeling

Integrating AI with causal inference methods (e.g., structural equation models, do-calculus) will enable predictions about the effects of genetic interventions, advancing personalized prevention.

7.4 Real-World Clinical Implementation

AI models must be validated in prospective clinical trials. Integration into electronic health records (EHRs) and decision support systems will facilitate early diagnosis and risk stratification.

7.5 Global Equity in Genomic Research

Expanding genomic databases to include diverse populations (e.g., African, South Asian) is essential to ensure that AI models are globally applicable and do not exacerbate health disparities.

7.6 Generative Models for Hypothesis Generation

Generative adversarial networks (GANs) and diffusion models can simulate genomic data, generating hypotheses about disease mechanisms and virtual patients for in silico trials.

Conclusion:

Decoding the genetic architecture of diabetes mellitus is a monumental challenge due to its polygenic nature, environmental interactions, and heterogeneity. Traditional genetic approaches have provided foundational insights but are insufficient to capture the full complexity of the disease. The integration of artificial intelligence and advanced genomic algorithms represents a paradigm shift in diabetes research.

AI models enable the analysis of massive, multi-dimensional datasets, uncovering non-linear patterns, predicting disease risk, prioritizing causal variants, and stratifying patients into biologically meaningful subtypes. When combined with multi-omics data, these tools elucidate the functional consequences of genetic variation and reveal dysregulated pathways in diabetes pathogenesis.

However, significant challenges remain, including data quality, model interpretability, ethical concerns, and clinical translation. Addressing these requires interdisciplinary collaboration among geneticists, computational biologists, clinicians, and ethicists.

The ultimate goal is precision medicine—tailoring prevention, diagnosis, and treatment to individual genetic profiles. With continued innovation, AI-powered genomics holds the promise of transforming diabetes care, reducing the global burden of disease, and improving patient outcomes. As we advance toward this future, robust validation, equitable access, and responsible use of AI will be paramount.

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MICROBIAL L-GLUTAMINASE: SOURCES, PRODUCTION AND APPLICATIONS

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Abstract:
L-Glutaminase is an important microbial amidohydrolase that catalyses the hydrolysis of L-glutamine to L-glutamic acid and ammonia and has attracted considerable attention due to its wide industrial and biomedical relevance. Microorganisms serve as efficient and sustainable sources of L-glutaminase, with diverse bacterial, fungal, and actinomycete species reported as potent producers. Advances in submerged and solid-state fermentation strategies have enabled improved enzyme yields and functional properties suitable for large-scale applications. Therapeutically, microbial L-glutaminase has shown promising antitumor and antiviral potential by selectively depleting extracellular glutamine required for the proliferation of malignant and infected cells. In the food industry, the enzyme plays a vital role in flavor enhancement of fermented products through increased glutamic acid formation, while its application in fine chemical synthesis, particularly for L-theanine production, has gained commercial importance. Additionally, L-glutaminase-based biosensors are increasingly used for monitoring glutamine and glutamate levels in clinical diagnostics and bioprocessing systems. This chapter discusses microbial sources, production strategies, and applications of L-glutaminase, highlighting its growing biotechnological significance.

Keywords: L-glutaminase; Antileukemic Activity; Food Industry Applications.

Introduction:

Enzymes are highly efficient biological catalysts produced by living organisms, and they are involved in all metabolic processes occurring inside and outside the cell. They increase the rate of a diverse set of chemical reactions essential for life (Devi *et al*., 2025). L-glutaminase is an amidohydrolase enzyme that catalyses the conversion of L-glutamine, in the presence of water, into L-glutamic acid and ammonia (El-Shora *et al*., 2025). The importance of L-glutaminase was

first reported by Alexander B. Gutman and Tsai-Fan (Gutman, 1963). El-Asmar and Greenberg (1966) reported that L-glutaminase from *Pseudomonas* sp. arrested the initial growth of several murine carcinomas but had little effect on the survival time of animals. Roberts. (1970) demonstrated that L-glutaminase from a Gram-negative, rod-shaped bacterium suppressed Ehrlich ascites carcinoma.

Microbial Sources of L-Glutaminase:

L-Glutaminase from Bacteria

Glutaminase production has been reported from many terrestrial bacteria such as *Proteus morganii*, *Escherichia coli*, *Acinetobacter* sp., *Pseudomonas* sp., and *Bacillus* sp. (Sabu, 2003). A few marine microorganisms are also known to synthesize L-glutaminase, including *Micrococcus luteus*, *Pseudomonas fluorescens*, *Vibrio costicola*, and *Beauveria bassiana* (Chandrasekaran, 1997). The production of L-glutaminase under submerged fermentation has been reported in *Bacillus licheniformis* (Cook, 1981), *Pseudomonas* sp. (Renu and Chandrasekaran, 1992), *Bacillus subtilis* (Sathish and Prakasham, 2010), and *Bacillus pasteurii* (Klein, 2002).

L-Glutaminase from Fungi

Fungi are potent producers of L-glutaminase. On an industrial scale, glutaminases are produced mainly by *Aspergillus* and *Trichoderma* species (El-Sayed, 2009). Submerged cultures of *Verticillium malthousei* (Imada, 1973), *Aspergillus sojae* (Yamamoto and Hirooka, 1974), *Aspergillus nidulans* (Saxena and Sinha, 1981), and *Penicillium notatum* (Shindia, 2007) have been used for L-glutaminase production.

L-Glutaminase from Actinomycetes

Actinomycetes are well recognized for producing a variety of bioactive compounds, many of which are valuable pharmaceuticals, agrochemicals, and industrial products such as enzymes. Marine actinobacteria exhibiting high L-glutaminase activity have been reported by several researchers (Teja *et al*., 2014). Abdallah *et al.* (2013) optimized the conditions for the purification of glutaminase from *Streptomyces avermitilis*.

Microbial Production of L-Glutaminase:

Submerged Fermentation

Microbial production of L-glutaminase is commonly carried out using submerged and solid-state fermentation techniques. Keerthi *et al.* (1999) isolated a salt-tolerant, extracellular glutaminase-producing Beauveria sp. BTMF S10 from marine sources, achieving a maximum activity of 46,900 U L^{-1} at pH 9.0 and an incubation temperature of 27°C. The medium was supplemented with 1% yeast extract, 9% sodium chloride, sorbitol, and 0.2% methionine. Kumar and Chandrasekaran (2003) reported an activity of 36,050 U L^{-1} using *Pseudomonas* sp. BTMS-51 supplemented with 2.0% L-glutamine and 1.0% D-glucose at pH 6.0 and 30°C.

Solid-State Fermentation

Glutaminase production using solid-state fermentation (SSF) has been investigated using various support materials. Wheat bran has been identified as a preferred support for enzyme production in SSF (Prabhu and Chandrasekaran, 1995). El-Sayed (2009) studied glutaminase production by *Trichoderma koningii* using wheat bran as the solid support and obtained a maximum activity of 45 U g^{-1} dry substrate. Optimal conditions included 70% initial moisture content, pH 7.0, supplementation with 1.0% D-glucose and 2.0% (w/v) L-glutamine, inoculation with 3 ml of culture, and incubation at 30°C for 7 days.

Commercial Production of L-Glutaminase:

Researchers at Kikkoman Corporation, Japan, have conducted pioneering work on L-glutaminase production. The company employed mutation and protoplast fusion techniques to improve L-glutaminase production by the koji mould *Aspergillus sojae* 116. A submerged fermentation process for producing thermostable L-glutaminase from *Cryptococcus nodaensis*, along with its purification, was developed and patented by the same group (Sato *et al*., 1999).

Applications of L-Glutaminase:

Therapeutic Applications

L-glutaminase has been explored for the treatment of lymphocytic leukemia due to its antileukemic activity. Leukemic cells rely on circulating L-glutamine as a metabolic precursor for nucleotide and protein synthesis; depletion of this amino acid results in selective inhibition of glutamine-dependent tumour cell proliferation (Abdelsayed *et al*., 2025). L-glutaminase derived from *Aspergillus niger* has demonstrated antitumour activity against human breast cancer cell lines (MCF-7), as confirmed by MTT assay (Dutt, 2014). L-glutaminase has also been proposed as a potential therapeutic agent for HIV infection. By lowering L-glutamine levels in tissues and serum, the enzyme reduces viral replication. A patented study reported the use of L-glutaminase from *Pseudomonas* sp. 7A to inhibit HIV replication in infected cells (Roberts, 2001).

Applications in the Food Industry

L-glutaminase is a key enzyme in enhancing the taste and aroma of fermented foods by increasing glutamic acid content. It imparts a pleasant and palatable taste to fermented foods such as soy sauce, sufu, and miso, and plays a major role in soy sauce fermentation. Flavour development occurs through the formation of amino acids produced by the degradation of L-glutamine by L-glutaminase (Sarada, 2013). Nakadai and Nasuno (1989) reported the use of glutaminase in soy sauce preparation. Their study demonstrated that large amounts of glutamic acid were formed in soy sauce by the addition of mycelia of *Aspergillus oryzae* or glutaminase from *Cryptococcus albidus.*

Manufacture of Fine Chemicals

Theanine is a major amino acid component of Japanese green tea and is known for its taste-enhancing properties. Increasing attention has been given to the physiological roles of theanine,

particularly due to its ability to suppress caffeine stimulation, enhance the effects of antitumour agents, and act as an antihypertensive agent. Tachiki *et al.* (1998) developed a strategy for the production of theanine from glutamate and ethylamine using a combined reaction of bacterial glutaminases and baker's yeast. Researchers at Taiyo Kagaku Co., Ltd., Japan, developed a method for the continuous production of theanine using immobilized *Pseudomonas nitroreducens* as a source of L-glutaminase. The process achieved a 95% theoretical yield of theanine based on the amount of glutamine consumed.

L-Glutaminase as a Biosensor

L-glutaminase has been widely used as a biosensor in hybridoma and mammalian cell cultures for monitoring glutamine levels (Sarada, 2013). The analysis of L-glutamine and glutamate levels in body fluids is important for clinical diagnostics and health monitoring. L-glutaminases are currently used either as free enzymes or immobilized on membranes as biosensors for monitoring glutamine and glutamate levels in fluids (Unissa *et al*., 2014). Botre *et al.* (1993) developed an L-glutaminase-based biosensor for the determination of glutamine and glutamate in pharmaceutical formulations.

Conclusion:

L-Glutaminase is an important microbial enzyme with wide-ranging applications in therapeutics, food processing, fine chemical synthesis, and biosensor development. Microorganisms provide efficient and sustainable sources for its production through submerged and solid-state fermentation, enabling high yields and desirable functional properties. Its antitumour and antiviral potential, along with its role in flavour enhancement of fermented foods, underscores its commercial and biomedical significance. Continued advances in strain improvement and process optimization are expected to further enhance the industrial and clinical applicability of microbial L-glutaminase.

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ARTIFICIAL INTELLIGENCE- DRIVEN PHYTOCHEMICAL RESEARCH: TRANSFORMING NATURAL PRODUCTS INTO NEXT-GENERATION DRUG CANDIDATES

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Abstract:
Phytochemicals are a rich and diverse source of bioactive compounds that have long aided drug research. Despite its great therapeutic potential, conventional phytochemical research has numerous challenges, including labor-intensive extraction and isolation procedures, low yields, poor reproducibility, limited target specificity, and inadequate pharmacokinetic and toxicological characterization. Recent advances in artificial intelligence (AI) have revolutionized natural product research by offering practical, data-driven answers to these limitations. AI and machine learning techniques speed up the discovery of new bioactive compounds by enabling rapid phytochemical profiling, automated GC-MS, LC-MS, and NMR data interpretation, and early dereplication of existing compounds. Furthermore, AI-based structure–activity relationship (SAR) and quantitative structure–activity relationship (QSAR) models enhance the prediction of biological activity, selectivity, and toxicity of complex natural products. AI-guided virtual screening, molecular docking, and protein–phytochemical interaction investigations facilitate rational target discovery and lead optimization. Additionally, ADMET prediction and AI-driven toxicity assessment improve safety evaluations and reduce late-stage drug development failures. AI combined with multi-target and network pharmacology methods enables a systems-level understanding of complex disease processes. For phytochemicals with polypharmacological effects, this is particularly crucial. Despite challenges with data accessibility, model interpretability, and regulatory approval, ongoing developments in explainable AI and ethical governance are increasing confidence in these technologies. All things considered, AI-assisted phytochemical research offers a strong and innovative basis for accelerating drug development and producing safer, more effective pharmaceutical compounds.

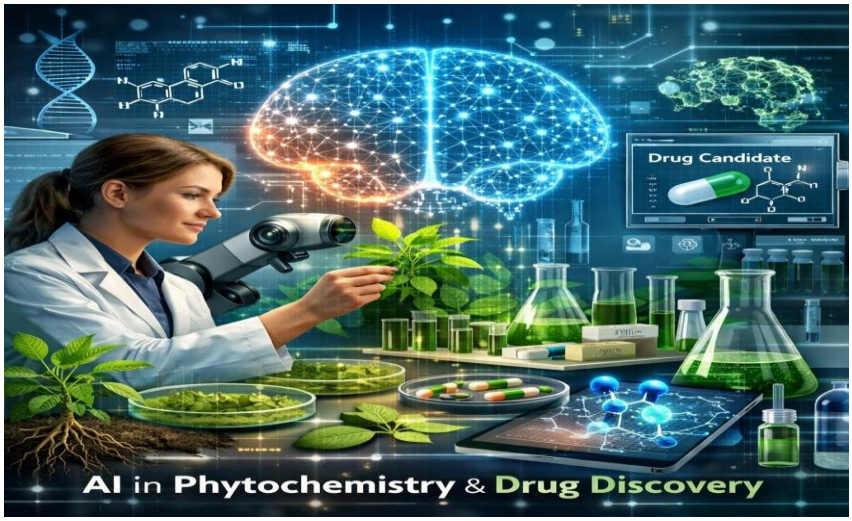
Keywords: Phytochemicals; Artificial Intelligence; Drug Discovery; SAR/QSAR Modeling; ADMET Prediction.

Introduction

Role of Phytochemicals in Modern Drug Discovery

Phytochemicals—bioactive compounds derived from plants—play an increasingly important role in modern drug delivery systems (DDS). Beyond their traditional use as therapeutic agents,

phytochemicals contribute significantly to improving drug formulation, targeting, and therapeutic efficacy. One major role of phytochemicals is as active pharmaceutical ingredients (APIs). Compounds such as curcumin, resveratrol, quercetin, and paclitaxel exhibit diverse pharmacological activities including anticancer, anti-inflammatory, antioxidant, and antimicrobial effects. However, many phytochemicals suffer from poor solubility, low bioavailability, and rapid metabolism. Modern drug delivery approaches help overcome these limitations. Phytochemicals are widely incorporated into novel drug delivery systems (NDDS) such as nanoparticles, liposomes, phytosomes, solid lipid nanoparticles, nanoemulsions, and polymeric micelles. These systems enhance solubility, protect phytochemicals from degradation, improve absorption, and allow controlled or sustained drug release. For example, curcumin-loaded nanoparticles show improved bioavailability and enhanced anticancer efficacy. Another important role of phytochemicals is in targeted and site-specific drug delivery. Certain plant-derived compounds exhibit affinity toward specific tissues or receptors, aiding targeted therapy and reducing systemic side effects. In cancer therapy, phytochemicals delivered via targeted nanocarriers can selectively accumulate in tumor tissues. Phytochemicals also act as natural excipients and delivery enhancers. Some compounds function as permeation enhancers, stabilizers, or antioxidants in formulations, improving drug stability and transport across biological barriers.[1,2]



Phytochemicals play a vital role in modern drug discovery by serving as rich sources of biologically active and structurally diverse compounds. Many successful drugs have originated from plant-derived molecules, demonstrating their therapeutic significance. With the integration of advanced techniques such as computational modeling, high-throughput screening, and AI-based approaches, phytochemicals continue to contribute as lead compounds and drug templates. Thus, they remain an indispensable component in the development of safe, effective, and innovative medicines.[3,4]

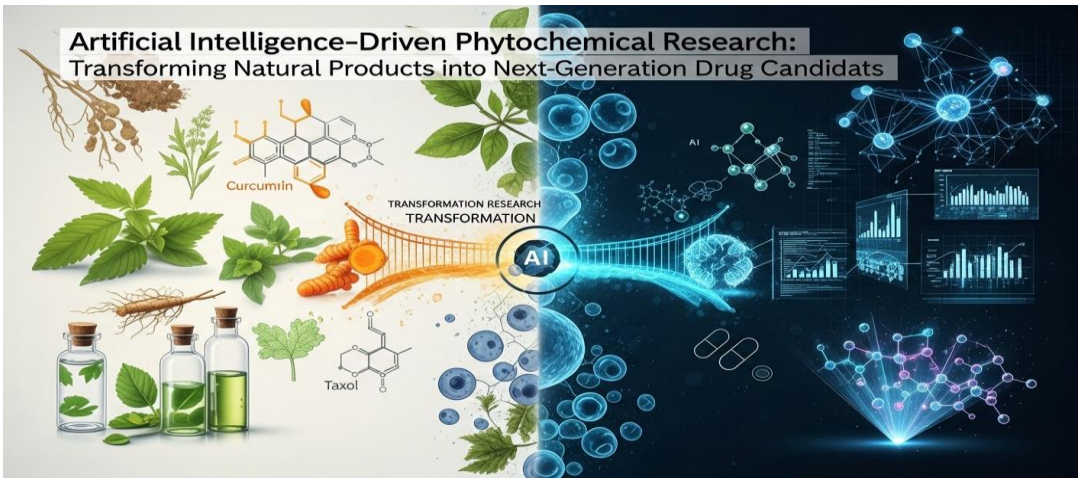
Limitations of Conventional Phytochemical Approaches

- Extraction and isolation processes are labor-intensive and time-consuming.
- Large amounts of plant material are needed, and known chemicals must be repeatedly isolated.
- Slow structural clarification by conventional analytical methods
- Absence of target-specific screening techniques In vitro activity and in vivo efficacy do not correlate well.
- Variability in phytochemical content brought on by seasonal and regional variables
- Inadequate comprehension of molecular mechanisms of action
- Inadequate pharmacokinetic and toxicological data restricting clinical translation Limited reproducibility and uniformity of results
- Low yield of bioactive compounds after isolation
- Difficulty in purifying complex mixtures of phytochemicals
- Limited sensitivity of conventional bioassays
- High cost associated with prolonged experimental procedures
- Challenges in scale-up and large-scale production
- Poor solubility and stability of many phytochemicals
- Limited availability of rare or endangered medicinal plants

Need for Artificial Intelligence in Natural Product Research

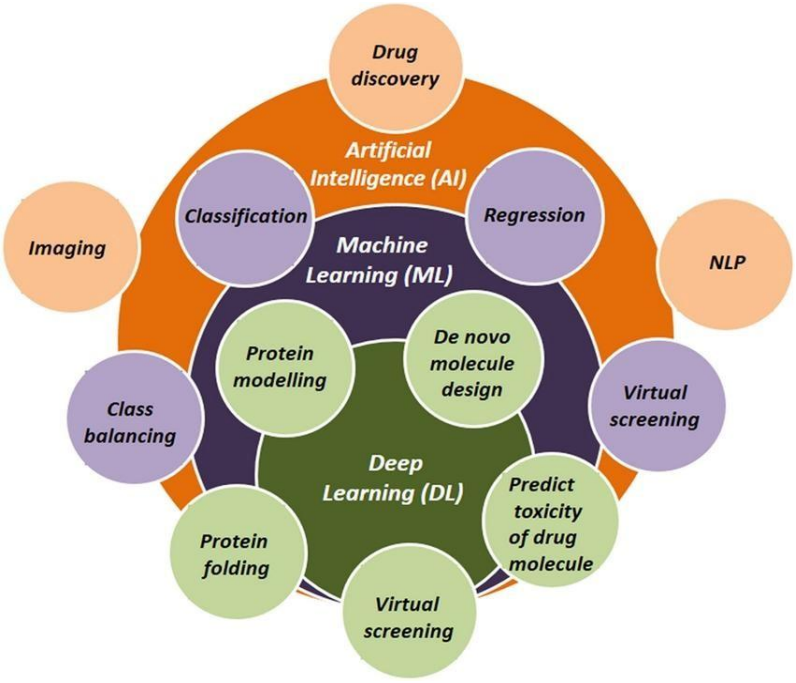
Natural product research, which offers a wide range of bioactive chemicals from plants, microbes, and marine sources, has long been a key component of drug development. However, sophisticated computational methods are required due to the intrinsic complexity, chemical variety, and time-consuming nature of traditional natural product research. In this regard, artificial intelligence (AI) has become a game-changing instrument that addresses major shortcomings of conventional approaches and speeds up the discovery and development of treatments based on natural products. The management and analysis of big, complicated datasets is one of the main requirements for AI in natural product research. Massive amounts of data are produced by contemporary methods including proteomics, metabolomics, genomics, and high-throughput screening [5,6]. These multidimensional datasets may be effectively processed, integrated, and interpreted by AI and machine learning algorithms, allowing for meaningful pattern recognition and knowledge extraction that are challenging to accomplish through manual analysis.AI is critically required for rapid identification and dereplication of natural compounds. Re-isolation of known compounds is a major challenge in natural product research. AI-assisted spectral analysis of GC–MS, LC–MS, and NMR data enables fast comparison with existing databases, facilitating early dereplication and allowing researchers to focus on novel and

promising compounds.Another important need for AI lies in prediction of biological activity and toxicity. AI models can predict pharmacological activity, selectivity, ADMET properties, and potential toxicity of natural compounds at an early stage. This reduces dependence on extensive in vitro and in vivo experiments, saving time, cost, and resources while improving the success rate of lead identification. [7-8]



In order to overcome the drawbacks of traditional, laborious phytochemical procedures, artificial intelligence is crucial for natural product development. AI makes it possible to quickly scan vast libraries of natural compounds, predict biological activity, and accurately identify therapeutic targets. It facilitates the prediction of pharmacokinetic and toxicological profiles, the dereplication of known chemicals, and the analysis of complicated datasets. AI increases the effectiveness of natural product-based medication development, speeds up lead identification, and lowers costs and failure rates by fusing computer modeling with experimental data.

Fundamentals of Artificial Intelligence in Drug Discovery



Overview of Artificial Intelligence and Machine Learning

Artificial Intelligence (AI) refers to the ability of computer systems to perform tasks that normally require human intelligence, such as learning, reasoning, problem-solving, decision-making, and pattern recognition. In recent years, AI has emerged as a transformative technology across multiple scientific disciplines, including healthcare, pharmaceutical sciences, and drug discovery. AI systems are designed to analyze large volumes of data, identify complex patterns, and generate predictive models that support informed decision-making.

Machine Learning (ML) is a major subset of AI that focuses on the development of algorithms capable of learning from data without being explicitly programmed. ML techniques enable computers to improve their performance through experience by identifying relationships between input variables and outcomes. Depending on the nature of learning, ML is broadly classified into supervised learning, unsupervised learning, and reinforcement learning. Common ML algorithms include linear regression, decision trees, support vector machines, k-nearest neighbors, and neural networks.

In pharmaceutical and life science research, AI and ML play a crucial role in handling complex biological and chemical datasets, such as genomic, proteomic, metabolomic, and chemical structure data. These technologies assist in virtual screening, target identification, lead optimization, prediction of drug–target interactions, and assessment of pharmacokinetic and toxicity profiles. Overall, AI and ML enhance research efficiency, accuracy, and innovation, making them indispensable tools in modern scientific and drug discovery processes.

Types of AI Techniques Used in Drug Discovery

- Artificial intelligence employs several techniques to support different stages of drug discovery.
- Machine Learning (ML) algorithms analyze chemical and biological data to predict drug–target interactions, biological activity, and toxicity.
- Deep Learning (DL), a subset of ML, uses neural networks to process complex data such as molecular structures and omics datasets with high accuracy.
- Natural Language Processing (NLP) is used to extract valuable information from scientific literature, patents, and clinical reports.
- Expert systems assist in decision-making by applying predefined rules and knowledge bases. Evolutionary algorithms and genetic algorithms help in lead optimization by generating and selecting improved molecular structures. Collectively, these AI techniques enhance speed, precision, and success rates in modern drug discovery.

Advantages of AI Over Traditional Computational Methods

- Effective management of extensive, intricate, and multidimensional datasets.
- Quicker analysis and forecasting than computational models based on rules Capacity to gain knowledge from data and gradually enhance performance

- Finding hidden and non-linear patterns in chemical and biological data
- Improved precision in anticipating drug-target interactions
- Quick virtual screening of extensive libraries of natural and chemical products
- Enhanced lead optimization and identification
- Improved pharmacokinetic and toxicity profile
- Prediction Decreased drug discovery time, expense and experimental failures Combining clinical, chemical, and multi-omics data for comprehensive analysis

AI in Phytochemical Profiling and Compound Identification

AI-Assisted Analysis of GC–MS, LC–MS, and NMR Data

In natural product research and drug discovery, analytical techniques such as Gas Chromatography–Mass Spectrometry (GC–MS), Liquid Chromatography–Mass Spectrometry (LC–MS), and Nuclear Magnetic Resonance (NMR) spectroscopy generate vast and complex datasets. Traditional interpretation of these data is often time-consuming, labor-intensive, and prone to human error, particularly when dealing with overlapping peaks, low-abundance metabolites, or complex mixtures.

Artificial Intelligence (AI) and Machine Learning (ML) offer advanced solutions to these challenges. AI algorithms can automatically process raw spectral data, deconvolute overlapping signals, and identify compounds with high accuracy. ML models can recognize patterns in mass spectra, predict chemical structures, and classify metabolites. In NMR analysis, AI assists in peak assignment, structure elucidation, and detection of minor components.

The integration of AI with GC–MS, LC–MS, and NMR not only accelerates metabolite identification but also improves reproducibility, reduces analytical errors, and facilitates high-throughput screening of complex natural extracts. This approach significantly enhances the efficiency of natural product-based drug discovery and phytochemical research.

Metabolomics and Dereplication Using AI

Metabolomics is the comprehensive study of small molecules, or metabolites, present within biological systems. In natural product research, metabolomics provides a detailed chemical profile of plant extracts, microbial cultures, or biological samples, enabling the identification of bioactive compounds and understanding of biochemical pathways. However, metabolomic datasets are typically large, complex, and multidimensional, making conventional analysis time-consuming and prone to errors.

Dereplication is a critical step in natural product discovery that involves the early identification of known compounds in complex mixtures. It prevents the repeated isolation of previously characterized metabolites, saving time, resources, and effort during drug discovery. Traditional dereplication relies heavily on manual comparison with spectral databases, which can be slow and limited in accuracy.

Artificial Intelligence (AI) and Machine Learning (ML) have transformed metabolomics and dereplication. AI algorithms can process large LC–MS, GC–MS, and NMR datasets, detect patterns, and predict molecular structures efficiently. Machine learning models can automatically match unknown spectra with reference databases, flag known compounds, and highlight novel metabolites for further investigation. Additionally, AI can integrate multi-omics data, providing insights into metabolic pathways, compound interactions, and biological activity.

The use of AI in metabolomics and dereplication offers several advantages:

- Accelerated identification of metabolites and bioactive compounds
- High-throughput screening of complex natural extracts
- Reduction in redundant isolation of known compounds
- Improved accuracy, reproducibility, and reliability of analysis
- Ability to detect minor or low-abundance metabolites that may be missed using traditional methods

3.3 Rapid Identification of Bioactive Phytochemicals:

The discovery of bioactive phytochemicals is a crucial step in natural product-based drug development. Traditional identification methods, relying on extraction, isolation, and bioassay-guided fractionation, are often time-consuming and labor-intensive. Artificial Intelligence (AI) and Machine Learning (ML) have revolutionized this process by enabling the rapid and efficient identification of active compounds. AI algorithms can analyze large chemical and biological datasets, predict potential bioactivity, and highlight promising lead molecules. Integration with analytical techniques such as GC–MS, LC–MS, and NMR allows automated spectral analysis, dereplication of known compounds, and structure prediction. Additionally, AI can prioritize compounds with favorable pharmacokinetic and toxicity profiles, reducing experimental failures. Overall, AI-assisted approaches significantly accelerate the discovery of novel phytochemicals while improving accuracy, reproducibility, and resource efficiency.

Additionally, AI can predict pharmacokinetic, toxicity, and drug-likeness properties, helping prioritize compounds with higher therapeutic potential. This approach not only accelerates the discovery of bioactive phytochemicals but also improves reproducibility, efficiency, and success rates in natural product research. By combining computational intelligence with experimental analysis, researchers can efficiently explore vast chemical diversity and identify promising leads for drug development.

AI-Based Structure–Activity Relationship (SAR) Modeling

Importance of SAR in Phytochemical Drug Design

Structure–Activity Relationship (SAR) analysis is a cornerstone in phytochemical drug discovery. It involves the systematic study of how the chemical structure of a compound influences its biological activity. By understanding SAR, researchers can identify the molecular

features essential for pharmacological effects and optimize lead compounds derived from natural products.

SAR aids in the following aspects of phytochemical medication design:

- Identification of Active Moieties: Determining which functional groups or structural motifs in a plant-derived compound are responsible for its therapeutic effect.
- Lead Optimization: Modifying chemical structures to enhance potency, selectivity, and pharmacokinetic properties while reducing toxicity.
- Predicting Biological Activity: SAR enables the rational prediction of the activity of new derivatives, saving time and resources in experimental testing.
- Guiding Synthetic Modifications: Provides a roadmap for designing semi-synthetic or fully synthetic analogs of natural products with improved efficacy.
- Understanding Mechanism of Action: Correlating structural changes with activity can reveal the molecular interactions between phytochemicals and their biological targets.

AI-Driven QSAR Models for Natural Products

A crucial computational technique in drug discovery is Quantitative Structure–Activity Relationship (QSAR) modeling, which makes it possible to predict biological activity based on chemical structure. Large, diversified datasets are typically difficult for classic QSAR algorithms to handle because to the increasing complexity of natural products. The predictive ability and effectiveness of QSAR models for natural products have been improved by artificial intelligence (AI), which has become a game-changing instrument.

Important Features of AI-Powered QSAR for Natural Products:

- Data Integration: AI algorithms can handle diverse data types, including chemical structures, physicochemical properties, and experimental bioactivity, allowing comprehensive modeling of natural compounds.
- Enhanced Predictive Accuracy: Machine learning techniques, such as random forests, support vector machines, and deep neural networks, improve the reliability of activity predictions for complex phytochemicals.
- Feature Extraction: AI can automatically identify important molecular descriptors or structural patterns that influence bioactivity, reducing manual bias in model development.
- Lead Prioritization: AI-driven QSAR enables rapid screening and ranking of natural product libraries to identify promising drug candidates.
- Discovery of Novel Scaffolds: By learning subtle structure–activity relationships, AI models can suggest modifications or entirely new scaffolds that may exhibit enhanced biological activity.
- Time and Cost Efficiency: Automation of predictive modeling accelerates early-stage drug discovery, reducing reliance on extensive experimental testing.

Prediction of Biological Activity and Selectivity

A crucial aspect in contemporary drug discovery is the prediction of biological activity and selectivity, which aids in the early identification of viable drug candidates. While selectivity prediction evaluates a molecule's capacity to act preferentially on the intended target with few off-target effects, biological activity prediction estimates the pharmacological action of a chemical on a particular biological target. Computational approaches such as structure–activity relationship (SAR) and quantitative structure–activity relationship (QSAR) models are widely used to correlate chemical structures with biological responses. The integration of artificial intelligence and machine learning has significantly improved these models by enabling the analysis of large and complex datasets and capturing non-linear relationships between molecular features and biological activity. Target binding, potency, and specificity are predicted by AI-based methods using molecular descriptors, docking studies, and virtual screening. Large compound libraries, including natural products, can be quickly screened using these techniques, which also assist in prioritizing compounds with superior selectivity profiles and high activity. Additionally, choosing safer and more efficient leads is supported by early prediction of ADMET

characteristics. In general, biological activity and selectivity prediction improves drug discovery efficiency by lowering experimental costs and time, increasing success rates, and directing the logical design of therapeutically effective and selective drug candidates.

AI-Guided Virtual Screening and Target Identification

Ligand-Based and Structure-Based Virtual Screening

Virtual screening, which enables the rapid identification of potential bioactive compounds from vast chemical libraries, is an essential computer technology in modern drug discovery. Depending on the type of information provided, virtual screening methods can be broadly classified as either structure-based virtual screening (SBVS) or ligand-based virtual screening (LBVS). Together, these strategies expedite lead optimization and identification.

LBVS, or ligand-based virtual screening: When the biological target's three-dimensional structure is unknown but known active ligands are known, ligand-based virtual screening is used. This strategy is predicated on the idea that substances with comparable physicochemical characteristics or chemical structures typically have comparable biological activity. Molecular descriptors, pharmacophore characteristics, and structure–activity interactions are all analyzed using LBVS techniques. Common LBVS techniques include similarity searching, pharmacophore modeling, and QSAR analysis. Similarity searching compares candidate compounds with known active molecules to identify structurally related analogues. Pharmacophore modeling defines the essential features required for biological activity, such as hydrogen bond donors, acceptors, hydrophobic regions, and aromatic rings. QSAR models

establish mathematical relationships between molecular descriptors and biological activity, enabling activity prediction for new compounds. LBVS is computationally efficient and particularly useful in early-stage screening of large natural and synthetic compound libraries. Virtual Screening Based on Structure (SBVS) When the three-dimensional structure of the target protein or receptor is known, usually through X-ray crystallography, NMR spectroscopy, or cryo-electron microscopy, structure-based virtual screening is used. The main goal of SBVS is to forecast how ligands will interact with the target's active or binding site. Molecular docking, which forecasts the binding orientation, interaction pattern, and binding affinity of ligands inside the target site, is the fundamental method of SBVS. Compounds are ranked according on their anticipated binding strength and stability using scoring methods. To account for protein flexibility and increase prediction accuracy, sophisticated SBVS techniques might include molecular dynamics simulations. SBVS facilitates logical drug design and lead optimization and offers comprehensive insights into ligand–target interactions.

Prediction of Protein–Phytochemical Interactions

A key component of contemporary drug discovery and natural product research is the prediction of protein–phytochemical interactions. As structurally varied and physiologically active substances originating from plants, phytochemicals mainly interact with particular proteins including enzymes, receptors, ion channels, and transporters to produce their therapeutic effects. To optimize phytochemicals as lead molecules, discover possible therapeutic targets, and clarify mechanisms of action, it is crucial to comprehend and anticipate these interactions. Enzyme assays, binding studies, and crystallographic analysis are examples of traditional experimental methods that are accurate but time-consuming and resource-intensive when researching protein–ligand interactions. For the quick and economical prediction of protein–phytochemical interactions, computational methods are now essential. Early in the research process, these techniques enable extensive screening of phytochemical libraries against several protein targets. One of the most popular methods for forecasting protein–phytochemical interactions is molecular docking. Docking studies predict the preferred binding orientation, important interactions, and binding affinity by simulating the binding of a phytochemical within the active or allosteric region of a target protein. To comprehend the stability and specificity of the interaction, hydrogen bonding, hydrophobic interactions, π–π stacking, and electrostatic forces are examined. Docking offers important information about how phytochemicals affect the function of proteins. Molecular dynamics (MD) simulations are used in addition to docking to investigate the stability and long-term behavior of protein–phytochemical complexes. MD simulations provide a more accurate depiction of biological environments by taking solvent effects and protein flexibility into account. By identifying conformational changes that affect binding strength and selectivity, these simulations aid in the validation of docking results. By

examining substantial databases of known protein–ligand interactions, machine learning and artificial intelligence techniques further improve interaction prediction. Using molecular descriptors and protein characteristics, AI models can forecast binding affinity, target specificity, and off-target interactions. Because phytochemicals frequently have multi-target or polypharmacological effects, this is very beneficial. Pathway analysis and network pharmacology are also supported by protein–phytochemical interaction prediction. Researchers can gain a better understanding of synergistic effects, therapeutic potential, and safety profiles by finding several protein targets for a single phytochemical. This all-encompassing strategy complements the multifaceted nature of herbal remedies.

AI in Multi-Target and Network Pharmacology

Because complex diseases including cancer, metabolic disorders, and neurodegenerative issues include several genes, proteins, and signaling pathways, the traditional "one drug–one target" approach is insufficient. The development of multi-target and network pharmacology, which concentrate on system-level interactions to manage this complexity, depends on artificial intelligence (AI). Artificial intelligence (AI) enables the prediction of multi-target interactions by analyzing large datasets of chemical structures, biological targets, and drug–target linkages. Machine learning and deep learning models allow a single drug, especially phytochemicals, to interact with several proteins simultaneously. This is particularly important in the study of natural goods, because compounds often exhibit polypharmacological effects.

AI helps create and analyze intricate networks that connect medications, targets, genes, processes, and illnesses in network pharmacology. AI-based network analysis finds important nodes, hubs, and pathways involved in the development of disease by combining data from proteomics, metabolomics, and genomes. This aids in understanding synergistic therapeutic effects and prioritizing targets. AI enhances safety and efficacy evaluation by supporting the study of drug combinations and herb-drug interactions. All things considered, combining AI with multi-target and network pharmacology offers a thorough, data-driven framework for comprehending intricate biological systems and creating safer, more potent treatment approaches.

AI in Pharmacokinetics, Toxicity, and Lead Optimization

AI-Based ADMET Prediction

AI helps create and analyze intricate networks that connect medications, targets, genes, processes, and illnesses in network pharmacology. AI-based network analysis finds important nodes, hubs, and pathways involved in the development of disease by combining data from proteomics, metabolomics, and genomes. This aids in understanding synergistic therapeutic effects and prioritizing targets. AI enhances safety and efficacy evaluation by supporting the study of drug combinations and herb-drug interactions. All things considered, combining AI with

multi-target and network pharmacology offers a thorough, data-driven framework for comprehending intricate biological systems and creating safer, more potent treatment approaches.

Early AI-based ADMET screening reduces late-stage failures by removing inappropriate compounds prior to expensive experimental testing. Additionally, it facilitates lead optimization by directing structural changes to enhance pharmacokinetic and safety characteristics. All things considered, AI-based ADMET prediction improves productivity, lowers development risk, and helps create safer and more potent medicinal medicines.

Toxicity and Safety Assessment of Phytochemicals

Despite their natural origin, phytochemicals' toxicity and safety evaluation is a crucial stage in their development as medicinal agents. Many phytochemicals can have harmful or hazardous effects depending on the dosage, length of exposure, and mode of administration, despite the fact that molecules originating from plants are frequently thought to be benign. Therefore, to guarantee their efficient and responsible use in medicine, rigorous safety evaluation is crucial. To determine acute, sub-chronic, and chronic toxicity as well as genotoxicity, hepatotoxicity, and reproductive toxicity, traditional toxicity assessment methods include in vitro experiments, in vivo animal studies, and clinical evaluation. These techniques, however, are expensive, time-consuming, and ethically dubious. In order to forecast toxicity profiles in the early stages of research, modern methodologies increasingly incorporate computational and AI-based tools. Toxic effects, off-target interactions, and dangerous dose ranges are identified by AI-based toxicity prediction models that examine chemical structures and biological data. These technologies lessen the need for lengthy animal testing and provide early risk assessment.

ADMET studies also shed light on accumulating hazards and metabolic pathways that affect safety.

AI-Guided Lead Optimization and Molecular Modification

With an emphasis on enhancing the effectiveness, selectivity, and safety of lead compounds, AI-guided lead optimization and molecular modification constitute a sophisticated phase of contemporary drug discovery. Compounds frequently need structural refinement to improve biological activity, pharmacokinetic characteristics, and developability after first hit or lead identification. Artificial intelligence (AI) offers strong instruments to direct this optimization process in a logical and effective way. AI models use structure–activity relationship (SAR) data analysis to find molecular characteristics that cause biological activity and unwanted characteristics. The effects of particular structural changes on potency, target selectivity, solubility, stability, and toxicity can be predicted by machine learning and deep learning algorithms. This makes it possible for researchers to focus on positive chemical alterations while avoiding changes that could result in decreased activity or more negative impacts.

AI-assisted methods for molecular modification recommend scaffold hopping, bioisosteric replacements, and functional group substitutions to produce optimal analogues. While maintaining the essential pharmacophore, generative AI models can create new molecular structures with enhanced characteristics. In natural product research, these methods are especially useful for optimizing complicated phytochemical scaffolds for improved drug-like action.

Challenges, Opportunities, and Future Perspectives

Data Availability and Model Interpretability

The successful use of artificial intelligence in drug development and pharmaceutical research depends critically on the availability of data and the interpretability of models. Large, excellent, and well-annotated datasets are crucial for AI models to produce precise and trustworthy predictions. Poor model performance and false results might result from inadequate, skewed, or inconsistent data. Data scarcity, a lack of standardized databases, and a lack of experimental annotations continue to be significant obstacles in natural product and phytochemical research, underscoring the need for better data sharing and curation. Model interpretability, or the capacity to comprehend and elucidate how an AI model makes a specific prediction, is equally crucial. Many sophisticated AI methods, particularly deep learning models, operate as "black boxes," making it challenging to understand how they make decisions. Researchers can find important chemical characteristics that cause biological activity, selectivity, or toxicity with the aid of interpretable AI models. This transparency facilitates hypothesis formulation, lead optimization, and risk assessment. To increase the trustworthiness and usefulness of AI models, strategies including feature importance analysis, attention mechanisms, and explainable AI (XAI) tools are being employed more frequently.

Regulatory and Ethical Considerations

Adoption of artificial intelligence (AI) in drug discovery, pharmaceutical research, and healthcare requires careful evaluation of ethical and regulatory issues. Ensuring adherence to ethical principles and regulatory norms is crucial for patient safety, scientific integrity, and public trust as AI-driven models increasingly impact decision-making processes. AI-based drug development technologies must exhibit transparency, repeatability, and dependability from a regulatory standpoint. Clear documentation of data sources, model development, validation processes, and performance measures is required by regulatory bodies. Predicting biological activity, toxicity, or clinical effects with AI requires scientific justification and experimental data. The absence of uniform legal frameworks for AI models continues to be problematic, especially for self-learning or adaptive systems.

Data privacy, informed consent, and bias reduction are examples of ethical issues. Large datasets, which may contain private biological or medical data, are frequently used by AI models. It is essential to guarantee data confidentiality and adherence to ethical standards.

Furthermore, biased or unrepresentative datasets may produce unfair results, underscoring the necessity of accountability and justice in AI model construction. Ethical requirements also include human monitoring and model interpretability. Opaque algorithms shouldn't be the only factor used to make decisions that impact patient health and medication safety. In conclusion, responsible, transparent, and reliable integration of AI into pharmaceutical research and drug development depends on strong regulatory supervision and ethical governance.

Future Scope of AI in Phytochemistry and Drug Discovery

Artificial intelligence (AI) has a very bright future in phytochemistry and drug discovery due to the growing need for more rapid, economical, and accurate drug development methods. AI is anticipated to play a revolutionary role in realizing the full medicinal potential of phytochemicals, which constitute a vast and mostly unexplored source of bioactive molecules. Through sophisticated analysis of metabolomics, genomes, and spectrum data, AI will greatly improve the discovery of novel bioactive chemicals in phytochemistry. Promising phytochemicals will be identified more quickly and with less redundancy thanks to automated dereplication, structural elucidation, and biological activity prediction. Targeted investigation of medicinal plants will be further guided by the integration of AI with large data resources and ethnopharmacological knowledge.AI is anticipated to promote network pharmacology, personalized medicine, and multi-target drug design in drug development. Lead selection will be enhanced and late-stage failures will be decreased with predictive models for activity, selectivity, and ADMET characteristics. Rational lead optimization and molecular alteration of intricate natural scaffolds to enhance drug-like characteristics will be made possible by generative AI and deep learning techniques.

Conclusion:

Phytochemicals remain a cornerstone of modern drug discovery due to their immense structural diversity and broad spectrum of biological activities. However, the limitations of conventional phytochemical approaches—including low yields, lengthy workflows, and poor clinical translation have restricted their full therapeutic potential. The integration of artificial intelligence has fundamentally transformed natural product research by enabling rapid phytochemical profiling, efficient dereplication, and reliable prediction of bioactivity, selectivity, toxicity, and pharmacokinetic behavior. AI-driven SAR/QSAR modeling, virtual screening, and multi-target analysis significantly improve lead identification, optimization, and rational drug design. Although challenges related to data quality, model interpretability, and regulatory acceptance persist, ongoing advances in explainable AI, data integration, and ethical frameworks continue to enhance confidence in these technologies. Overall, AI-assisted phytochemical research represents a robust, efficient, and future-ready paradigm for the development of safe, effective, and innovative therapeutics in the modern pharmaceutical landscape.

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