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Natural Deep Eutectic Solvents (NADES): A Green Revolution in Bioactive Compound Extraction

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ABSTRACT:

The use of Natural Deep Eutectic Solvents (NADES) to extract bioactive substances from natural sources is becoming more and more popular as an environmentally friendly, biodegradable, and effective substitute for conventional organic solvents. NADES are made up of mixtures of natural metabolites such sugars, organic acids, amino acids, and salts based on choline. They have special qualities like low volatility, non-flammability, high thermal stability, and polarity that may be adjusted. These qualities enable them to be especially successful in solubilizing a variety of phytochemicals, including phenolic acids, terpenoids, alkaloids, flavonoids, and saponins. This paper discusses the formulation methods, molecular interactions, and physicochemical properties of Natural Deep Eutectic Solvents (NADES), focusing on their application in environmentally friendly extraction techniques. It explores the synergistic integration of NADES with modern methods such as enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE). These methods demonstrate superior chemical preservation, reduced energy consumption, and enhanced extraction efficiency. NADES also improve the stability and solubility of bioactive compounds, making them ideal solvents for pharmaceutical and nutraceutical applications. Aligning with the principles of green chemistry, NADES offer a safer, more eco-friendly, and economically viable approach to recovering bioactive compounds. This analysis underscores their growing significance in the sustainable extraction of natural products and their potential for large-scale green processing in the future.

Key word: Natural Deep Eutectic Solvents (NADES),Green solvents, Bioactive compound extraction, Plant secondary metabolites, Phytochemical extraction, Herbal extraction, Green chemistry.

Introduction:

The extraction of bioactive compounds from natural sources, especially medicinal and aromatic plants, has long been an essential process in the pharmaceutical, nutraceutical, and cosmetic industries. These bioactive compounds—including flavonoids, alkaloids, phenolic acids, terpenoids, and glycosides—are recognized for their therapeutic properties, which include antioxidant, anti-inflammatory, antimicrobial, and anticancer activities[18].

Traditionally, the extraction of phytoconstituents heavily relies on organic solvents such as methanol, ethanol, acetone, and chloroform. While these solvents are effective, they have significant drawbacks: they are toxic, volatile, flammable, non-biodegradable, and pose serious health and environmental risks[19].Additionally, the energy-intensive processes involved in their use and recovery contradict the principles of green chemistry.

There is an increasing global interest in green solvent systems that are safe, sustainable, and efficient, in response to various challenges. Among the different alternatives being explored—such as supercritical fluids, ionic liquids, and bio-based solvents—Natural Deep Eutectic Solvents (NADES) have emerged as a particularly promising class of eco-friendly solvents. These solvents are both cost-effective and biodegradable [20,21].

NADES, or Natural Deep Eutectic Solvents, are typically made up of two or more naturally occurring compounds. These can include sugars (such as glucose and sucrose), amino acids (like proline and glycine), organic acids (such as citric, malic, and lactic acids), or choline chloride. These components are mixed in specific molar ratios and interact through extensive hydrogen bonding. As a result, they form a eutectic system with a significantly lower melting point than that of the individual components [22].

NADES (Natural Deep Eutectic Solvents) are especially well-suited for bioactive extraction due to their biocompatibility, adjustable polarity, and non-volatility. These properties enable them to dissolve both polar and non-polar compounds effectively [23]. Additionally, their low toxicity and biodegradability ensure safe handling, making them ideal for direct use in pharmaceutical and food applications without the need for complete solvent removal.

Furthermore, NADES often replicate the intracellular solvent systems of plants, providing a more "natural" environment for extracting secondary metabolites while preserving their bioactivity and structure [24].

A key benefit of NADES is their adaptability and versatility. The physicochemical properties of NADES, including viscosity, polarity, and solubility, can be precisely adjusted by altering the composition and water content, facilitating customization for specific extraction targets [25].

Furthermore, their high thermal and chemical stability enables integration with contemporary extraction technologies, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), and pressurized liquid extraction (PLE).

When combined with Naturally Derived Solvents (NADES), various techniques have been shown to increase yield, reduce processing time, lower energy consumption, and maintain the structural integrity of bioactive compounds [26,27]. This leads to more efficient and sustainable extraction processes. Additionally, NADES have demonstrated the potential to enhance the solubility, stability, and bioavailability of poorly soluble natural compounds. This makes them not only effective solvents but also promising carriers or formulation components in drug delivery systems.

Although there are many advantages, certain limitations still exist, such as high viscosity and difficulties in recovery and recyclability. However, recent innovations are addressing these issues. Solutions include the incorporation of water or co-solvents, the use of low-viscosity Natural Deep Eutectic Solvents (NADES), and membrane-assisted separation techniques [28,29].

History and Evaluation:

1.Origins: From Deep Eutectic Solvents (DES) to NADES

In 2003, Abbott and colleagues introduced the concept of Deep Eutectic Solvents (DES). When choline chloride was mixed with substances such as urea, they discovered that the resulting liquids had melting points significantly lower than those of the individual components[1].

The investigation of DES variations with organic salts and metal halides (Classes I–IV) was expanded in the early 2000s, specifically in 2001.

2.The Birth Of NADES (2011)

Natural Deep Eutectic Solvents (NADES) are mixtures of natural metabolites, such as sugars, amino acids, organic acids, and choline derivatives, that become liquid at room temperature. These solvents were introduced by Choi, Verpoorte and their colleagues at Leiden University in 2011[2].By using heating or vacuum evaporation methods, over 100 combinations were created (e.g., sugar–sugar–acid, choline chloride with organic acids). The strong hydrogen-bond (H-bond) networks exhibited by these NADES enhanced the solubility of biomolecules. Based on metabolomics, NADES likely occur naturally in plants and serve as co-solvent phases alongside lipids and water[3,4]

3. Natural Roles In Organisms: Biological Insight

Natural Deep Eutectic Solvents (NADES) serve several important functions:

1. Metabolic Solvents: They facilitate biosynthesis and help preserve metabolites that have low solubility in water.
2. Desiccation Protectants: These substances stabilize water molecules and prevent crystallization, allowing organisms such as cacti and cold-tolerant insects to survive in extreme conditions.
3. Stabilisers: They are effective at preserving proteins, DNA/RNA, and pigments, such as carthamin [5,6]
4. Technological Evolution And Application:

Functional Tunability: Natural Deep Eutectic Solvents (NADES) exhibit a range of properties, from hydrophilic (water-attracting) to lipophilic (fat-attracting), depending on their components, which may include sugars, acids, and polyols. NADES are also recognized for their low volatility, non-flammability, biodegradability, low toxicity, and cost-effectiveness, making them exemplary green solvents. Common techniques for preparing NADES include heating, vacuum evaporation, freeze-drying, and ultrasound-assisted synthesis [9,10].

Definition and Concept of NADES:

Natural Deep Eutectic Solvents (NADES) are a category of environmentally friendly solvents created by combining two or more natural, non-toxic substances. Usually, these mixtures consist of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). When these components are mixed in a specific molar ratio, they form a liquid that has a melting point much lower than that of the individual components.

Concept of NADES:

1.Origin and Background:

- NADES, or natural deep eutectic solvents, were first introduced by Paiva et al. and the Verpoorte group. They represent an evolution of ionic liquids and traditional eutectic solvents.
- NADES mimic the intracellular environment found in plants and organisms, which may help explain how some poorly soluble compounds are stored within cells

2.Components:

Typical constituents include:

- Sugars (glucose, fructose)
- Organic acids (citric acid, lactic acid)
- Amino acids
- Choline chloride
- Urea

3.Formation:

NADES are formed by:

- Hydrogen bonding between donor and acceptor molecules
- Mixing at a defined molar ratio
- Gentle heating (typically ≤ 60°C)

This results in a stable, homogeneous liquid phase.

4.Advantage:

- Non-toxic and biodegradable

- Derived from renewable resources
- Adjustable polarity and viscosity
- Enhanced solubilization of polar and non-polar bioactive compounds
- Compatible with green chemistry principles

Classification of NADES:

Type	Example components	Description
Sugar-based	Glucose, sucrose +choline chloride	Good for polar bioactive extraction
Organic acid- based	Citric acid, malic acid+ urea	Acidic environment, useful for alkaloid and phenolic compounds
Amino acid- based	Proline, glycine + lactic acid	Biocompatible, often used in biocatalysis
Alcohol- based	Glycerol, sorbitol + choline chloride	Low toxicity, hydrophilic
Mixed NADES	Combination of above types	Tailored polarity and functionality

Physicochemical properties of NADES:

- **i.** Viscosity: Generally high, but can be reduced by adding water (carefully to avoid disruption of eutectic structure)
- **ii.** Polarity: Tunable based on components
- **iii.** Density: Higher than water (1.1–1.3 g/cm^3)
- **iv.** Melting Point: Much lower than individual components
- **v.** Conductivity: Low, but can be improved with ion-containing NADES

Preparation method of NADES:

There are four common methods to prepare NADES:

a) Heating and stirring method
- Simple and widely used
- Components are mixed in specific molar ratios and heated (up to 80°C)

b) Vacuum evaporation method
- Used when components are not miscible
- Solvent (usually water or ethanol) is removed under reduced pressure

c) Freeze-drying (lyophilization)
- Used to maintain thermal stability of heat-sensitive compounds

d) Grinding method
- Mechanical mixing at room temperature (eco-friendly, solvent-free)

➢ Limitation of NADES:

While promising, NADES still have some limitation

a) High viscosity can reduce extraction efficiency or complicate filtration.
b) Limited volatility makes them hard to remove from final product.
c) Lack of standardization for toxicity and biodegradability profiles.
d) Some NADES may interfere with analytical detection techniques (e.g., UV spectroscopy).

➢ Extraction techniques using NADES:

NADES are used as alternatives to conventional solvents in various extraction methods. The choice of technique depends on the target compound, matrix, and physical properties of the NADES used.

1. Conventional Heat-Assisted Extraction (HAE)
Concept:
* Plant material is mixed with NADES and gently heated (typically 40–80°C).
* Heating reduces viscosity and enhances solubility of compounds.
Advantages:
* Simple, cost-effective
* Enhanced mass transfer
* Good for thermally stable compounds
Limitations:
* Not suitable for thermolabile compounds [39].
2. Ultrasound-Assisted Extraction (UAE)
Concept:
* Uses ultrasonic waves to disrupt plant cells, allowing NADES to penetrate and extract target molecules more efficiently.
Key Features:

* Lower temperature (\~30–50°C)
* Short extraction time (minutes)
* High yield, even for heat-sensitive compounds

Advantages:
* Reduces solvent use
* Energy-efficient
* Suitable for polyphenols, flavonoids, and alkaloids [40].

3. Microwave-Assisted Extraction (MAE)

Concept:
* Microwaves heat the solvent and plant matrix uniformly.
* Disrupts plant cell walls rapidly, enhancing NADES-based extraction.

Advantages:
* Rapid extraction (seconds to minutes)
* Higher selectivity and purity
* Reduced energy and solvent consumption

Considerations:
* NADES must be microwave-absorbent
* Viscosity of NADES may need adjustment with water [41].

4. Stirring-Assisted Extraction (SAE)

Concept:
* Mixing powdered plant material with NADES under magnetic or mechanical stirring at ambient or mild temperature.

Advantages:
* Simple, energy-efficient
* Low degradation risk for sensitive molecules

Best For:
* Extraction of polar compounds like tannins, saponins [42].

5.Pressurized Liquid Extraction (PLE)
(Also called Accelerated Solvent Extraction)

Concept:
* NADES are used under elevated temperature (50–150°C) and pressure (10–15 MPa) to extract compounds from solid matrices.

Advantages:
* Efficient for large-scale operations
* High recovery and purity

Disadvantages:
* Requires special equipment
* May degrade thermolabile compounds [43].

6. Enzyme-Assisted Extraction (EAE)

Concept:
* NADES combined with cell-wall degrading enzymes (e.g., cellulase, pectinase) to facilitate release of intracellular bioactives.

Applications:
* Gentle technique for functional foods, pharmaceuticals
* Enhances flavonoid and phenolic extraction

Note:
* NADES must be enzyme-compatible (low toxicity and moderate pH) [44].

7. Green Solvent Hybrid Extraction (with NADES + Water or Ethanol)

Concept:
* NADES are often diluted with water or ethanol to reduce viscosity and improve penetration.
* Tailors solvent polarity
* Increases efficiency for both hydrophilic and lipophilic compounds.

Factors affecting extraction efficiency:

1. Nature and Composition of NADES

a) Type of Components (HBA and HBD):
* The hydrogen bond acceptor (e.g., choline chloride) and donor (e.g., organic acids, sugars, alcohols) determine the polarity, solubility, and extraction power.
* A proper match ensures stronger interaction with the target compound [30].

b) Molar Ratio of Components:

* Changes in the HBA: HBD molar ratio affect the viscosity, polarity, and pH.
* Example: A 1:2 choline chloride: citric acid NADES may extract better than a 1:1 ratio for phenolics [31].

2. Viscosity of NADES

* High viscosity reduces mass transfer between the plant material and the solvent.
* Lower viscosity (e.g., by adding 10–30% water) improves diffusion and extraction kinetics.
* However, too much water may break the eutectic structure and reduce solvating power [32].

3. Water Content in NADES

* Water acts as a viscosity reducer and polarity modulator.
* Optimal water content (~20–40%) can improve extraction; excessive water leads to dilution of H-bonding network [33].

4.Extraction Temperature

* Higher temperature reduces NADES viscosity and improves diffusion rate, enhancing extraction.
* However, thermal degradation of heat-sensitive compounds may occur.
* Common range: 30–80°C, depending on target compound [34].

5. Extraction Time

* Sufficient time allows solvent penetration and solubilization of bioactives.
* However, excessively long extraction may cause oxidation or degradation of sensitive compounds.
* Optimized extraction time depends on the method (e.g., 30–60 min for UAE, 2–4 hours for SAE) [35].

6.Solid-to-Liquid Ratio (S/L ratio)

* Determines the solvent-to-sample contact surface.
* A higher solvent ratio generally enhances extraction yield, but after a certain point, returns diminish.
* Common ratio: 1:10 to 1:30 (g/mL) [36].

7. Particle Size of Plant Material

* Smaller particles increase surface area, improving solvent penetration and mass transfer.
* Too fine particles may lead to clogging and filtration difficulty due to high viscosity of NADES.

8. Type of Extraction Technique Used

* The physical mechanism used (ultrasound, microwave, stirring, pressurization) affects the rate and yield.
* For example, UAE and MAE offer better efficiency and shorter extraction times than traditional methods [37].

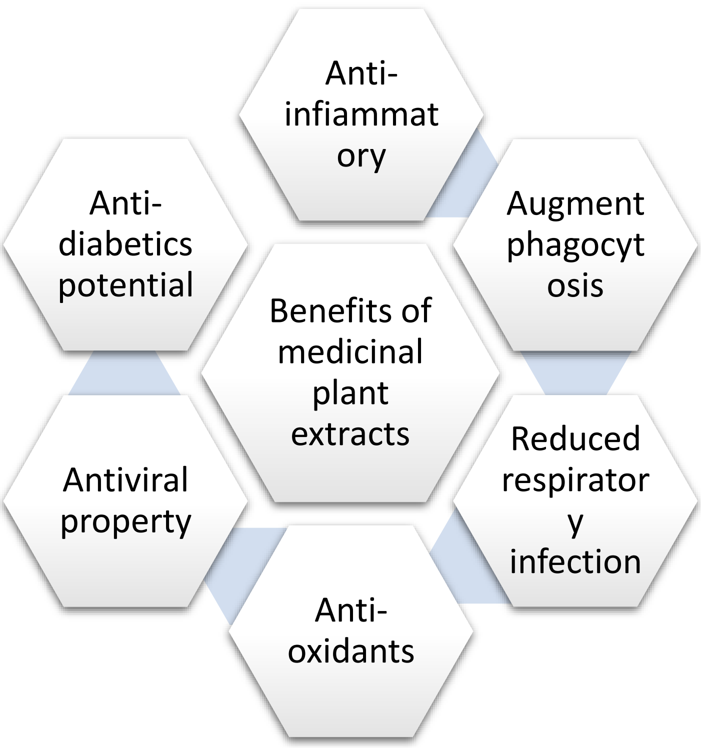
9. Polarity Match Between NADES and Target Compound

* NADES should have similar polarity to the target molecule (like-dissolves-like).
* For example, sugar-based NADES are more polar (ideal for polyphenols), while alcohol-based NADES suit lipophilic compounds.

10. pH of NADES System

* pH affects the stability and solubility of target compounds, especially for phenolics and alkaloids.
* Acidic NADES (e.g., citric acid-based) are better for acid-stable compounds.

FIG: schematic representation of the potential beneficial effects of medicinal plant extraction in the prevention and treatment of many diseases [45].



Conclusion:

Natural Deep Eutectic Solvents (NADES) are increasingly recognised as innovative, eco-friendly, and efficient alternatives to traditional organic solvents for extracting bioactive compounds from natural sources. Based on the principles of green chemistry and sustainability, NADES provide a viable method for extracting valuable phytochemicals while minimising environmental impact and enhancing safety.NADES have a unique ability to create hydrogen bonding networks between hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs). This results in solvents with low melting points, high thermal and chemical stability, and adjustable polarity. These characteristics enable NADES to effectively extract a wide range of compounds, including highly polar phenolics and flavonoids, as well as less polar alkaloids and terpenoids, while preserving the stability and bioactivity of the extracted compounds.

This review has covered:

1) The classification and preparation techniques of NADES.
2) Their physicochemical characteristics (such as viscosity, polarity, and density).
3) The various extraction methods employed (e.g., UAE, MAE, SAE, HAE, PLE).

And the key influencing factors affecting extraction efficiency, including viscosity, temperature, water content, NADES composition, extraction time, and method used.

- Future Outlook:

The future of NADES lies in their continued optimization and adaptation to specific industrial applications. Key areas of focus include:

- ➢ Designing NADES with low viscosity and high selectivity.
- ➢ Integrating NADES in large-scale extraction and purification workflows.
- ➢ Developing greener downstream processes to recover both NADES and bioactive compounds efficiently.
- ➢ Evaluating the environmental footprint and toxicology of long-term use.
- ➢ Combining NADES with advanced technologies such as supercritical fluid extraction, membrane separation, and nanotechnology

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