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Applications of Deep Eutectic Solvents (DESs) in the Extraction of Bioactive Compounds:

A Comprehensive Review on Physicochemical Dynamics, Optimization Strategies, and Industrial Sustainabil

تطبيقات المذيبات سهلة الانصهار العميقة (DESs) في استخلاص المركبات النشطة بيولوجياً:

مراجعة شاملة للديناميكيات الفيزيائية والكيميائية، استراتيجيات التحسين، والاستدامة الصناعية

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Abstract:

To overcome the environmental and operational limitations associated with volatile organic solvents, green chemistry research has increasingly turned toward customized fluid systems. Deep Eutectic Solvents (DESs) and their naturally derived counterparts (NADESs) represent a versatile class of designer media synthesized through the molecular pairing of hydrogen bond acceptors (HBAs) and donors (HBDs). These mixtures exhibit distinct physicochemical profiles, including negligible vapor pressures, modular solvation capacities, tunable viscosities, and high thermal endurance. This review offers a critical

المستخلص:

للتغلب على القيود البيئية والتشغيلية المرتبطة بالمذيبات العضوية المتطايرة، اتجهت أبحاث الكيمياء الخضراء بشكل متزايد نحو أنظمة السوائل المصممة خصيصًا. تمثل المذيبات اليوتكتيكية العميقة (DESs) ونظائرها المشتقة طبيعيًا (NADESs) فئة متعددة الاستخدامات من الوسائط المصممة، والتي يتم تصنيعها من خلال الاقتران الجزيئي لمستقبلات ومانحات الروابط الهيدروجينية (HBAs و HBDs). تتميز هذه الخلائط بخصائص فيزيائية وكيميائية فريدة، بما في ذلك ضغوط بخار ضئيلة، وقدرات إذابة قابلة للتعديل، ولزوجة قابلة للضبط، ومقاومة حرارية عالية. تقدم هذه المراجعة تقييمًا نقديًا للتصنيفات الهيكلية للمذيبات اليوتكتيكية العميقة،

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assessment of DES structural classifications, formulation methodologies, and solubilization mechanisms driven by supramolecular forces, hydrogen bonding networks, and hydrophobic interactions. Additionally, process intensification protocols—such as ultrasound, microwave, and pressurized liquid assistance—are systematically analyzed alongside Response Surface Methodology (RSM) parameters. The paper further evaluates downstream solute separation, solvent regeneration, ecotoxicological boundaries, and commercial scalability metrics to establish a foundation for industrial biorefinery integration.

ومنهجيات التركيب، وآليات الإذابة المدفوعة بالقوى فوق الجزيئية، وشبكات الروابط الهيدروجينية، والتفاعلات الكارهة للماء. بالإضافة إلى ذلك، يتم تحليل بروتوكولات تكثيف العمليات - مثل الموجات فوق الصوتية، والميكروويف، والمساعدة السائلة المضغوطة - بشكل منهجي جنبًا إلى جنب مع معايير منهجية سطح الاستجابة (.RSMكما تقيّم الورقة فصل المذاب في المراحل اللاحقة، وتجديد المذيب، والحدود السمية البيئية، ومقاييس قابلية التوسع التجاري لوضع أساس لتكامل المصافي الحيوية الصناعية.

Keywords:
Deep Eutectic Solvents; Green Extraction; Bioactive Compounds; Hydrogen Bond Donors; Supramolecular Chemistry; Sustainability; Process Intensification.

الكلمات المفتاحية:
المذيبات اليوتكتيكية العميقة؛ الاستخلاص الأخضر؛ المركبات النشطة بيولوجيًا؛ مانحي الروابط الهيدروجينية؛ الكيمياء فوق الجزيئية؛ الاستدامة؛ تكثيف العمليات.

1. Introduction

The global demand for high-value bioactive metabolites—including polyphenolic complexes, flavonoids, alkaloids, carotenoids, and essential lipids—has accelerated rapidly across the pharmaceutical, nutraceutical, cosmeceutical, and functional food sectors [1]. Traditional recovery of these bioactive constituents from plant biomass, marine matrices, and agricultural residues relies predominantly on volatile organic solvents (VOCs) such as hexane, dichloromethane, methanol, and ethyl acetate [2]. Although VOCs exhibit strong solvation capabilities, their commercial utilization incurs significant ecological, toxicological, and financial liabilities. VOCs are generally flammable, atmospheric pollutants, hazardous to human health, and derived from petrochemical refining [3]. Their environmental discharge fuels greenhouse gas accumulation and industrial waste challenges, directly conflicting with modern Green Chemistry metrics [4].

To establish cleaner extraction paradigms, functional alternatives have been extensively investigated. Ionic Liquids (ILs) emerged as early low-volatility substitutes; however, complex multi-step synthesis protocols, elevated production expenses, poor biodegradability, and persistent aquatic toxicity restricted their large-scale industrial implementation [5]. A pivotal paradigm shift occurred with the characterization of Deep Eutectic Solvents (DESs) by Abbott and co-workers [6]. DESs are formulated by combining two or more pure crystalline components—a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD)—at specific stoichiometric ratios. The resulting eutectic liquid exhibits a significant depression in freezing point relative to an ideal mixture, maintaining a fluid state over broad temperature ranges [7].

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When synthesized exclusively from primary cellular metabolites—such as amino acids, organic acids, polyols, sugars, and choline derivatives—these platforms are designated as Natural Deep Eutectic Solvents (NADESs) [8]. DESs and NADESs provide key operational benefits: near-zero vapor pressure, high thermal stability, non-flammability, modular solvation capacities, and customized polarities [9]. Furthermore, their preparation achieves 100% atom economy without generating secondary reaction byproducts or requiring costly purification protocols [10]. This comprehensive review delivers a critical analysis of DES structural classification, preparation methods, physicochemical kinetics, process intensification pathways, downstream recovery systems, and techno-economic criteria governing industrial scale-up.

2. Classification, Synthesis, and Structural Dynamics of DESs

2.1 Classification Framework of Deep Eutectic Solvents

DES systems are broadly categorized into five primary structural classes based on the chemical nature of their HBA and HBD constituents, as detailed in Table 1 [6, 11].

Type	General Formula	Representative Components	Primary Characteristics & Applications	Ref.
Type I	$Cat^+ X^- + z MCl_x$	Choline chloride + $ZnCl_2$, $FeCl_3$	Lewis acid systems; non-aqueous catalysis, electrodeposition.	[6]
Type II	$Cat^+ X^- + z MCl_x \cdot y H_2O$	Choline chloride + $CrCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$	Hydrated metal complexes; moisture-resilient, industrial plating.	[6]
Type III	$Cat^+ X^- + z RZ$	Choline chloride + Urea, Glycerol, Lactic Acid	Dominant extraction media; biodegradable, highly biocompatible.	[7, 8]
Type IV	$MCl_x + z RZ$	$ZnCl_2$ + Urea, Acetamide, Ethylene glycol	Metal halide/HBD complexes; specialized organic synthesis.	[11]
Type V	Non-ionic HBA + HBD	Thymol + Menthol, Decanoic acid + Lidocaine	Hydrophobic DESs; immiscible with water, extracts non-polar bioactives.	[12]

Among these categories, Type III DESs—particularly those utilizing Choline Chloride (ChCl) paired with natural HBDs such as organic acids (citric, malic, lactic), polyols (glycerol, ethylene glycol), or carbohydrates (glucose, fructose)—are widely applied in bioactive extraction due to low cost, safety, and rapid environmental degradation [8, 13].

2.2 Synthesis Protocols and Quality Management

The preparation of DES media relies on direct, atom-economical blending procedures that avoid organic solvents [14]:

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- **Heating and Agitation Method:** The standard laboratory and industrial strategy. Precise molar ratios of solid HBA and HBD are combined under continuous mechanical stirring at controlled temperatures (50 °C – 90 °C) until a transparent, homogeneous liquid is formed [8].
- **Vacuum Evaporation / Freeze-Drying:** Components are co-dissolved in an aqueous phase, homogenized, and subsequently subjected to rotary vacuum evaporation or lyophilization to yield a pure DES matrix [9].
- **Mechanochemical Grinding:** Solid precursor crystals are co-ground at room temperature using mortar systems or ball mills until spontaneous liquefaction occurs, serving as a low-thermal route for heat-sensitive constituents [10].

2.3 Supramolecular Network and Eutectic Depression Mechanisms

The liquidity of DES systems stems from an extensive supramolecular hydrogen-bonding network established between the HBA anion (e.g., Cl^- of Choline Chloride) and the donor functional groups (hydroxyl, carboxyl, amine) of the HBD [15]. Charge delocalization across the hydrogen-bonded complex reduces the lattice energy of the starting components, leading to a significant depression in the experimental melting point (T_m) relative to ideal liquid mixture behavior ($T_{m,ideal}$) [16].

3. Physicochemical Properties Influencing Bioactive Extraction

3.1 Viscosity Kinetics

Many baseline DES formulations exhibit elevated viscosities ($\eta > 100$ mPa·s at 25 °C), caused by dense hydrogen-bonding networks and steric hindrance [17]. High viscosity impedes mass transport during extraction by lowering solute diffusion coefficients according to the Stokes-Einstein expression:

$$D = (k_B \cdot T) / (6 \cdot \pi \cdot \eta \cdot r)$$

Where D is the diffusion coefficient, k_B is the Boltzmann constant, T is absolute temperature, η is dynamic viscosity, and r is the hydrodynamic radius of the solute [17]. To lower diffusion resistance, extractions are typically conducted at moderate thermal regimes (40 °C – 70 °C) or with controlled hydro-dilution (10% – 30% v/v water), which lowers medium viscosity while retaining core supramolecular structures [18].

3.2 Density and Polarity Customization

DES densities generally fall between 1.05 g/cm³ and 1.35 g/cm³ depending on molecular packing and component selection [19]. Solvent polarity is highly tunable; by altering HBD structures (e.g., transitioning from hydrophilic carbohydrates to organic acids or hydrophobic fatty acids), the empirical polarity parameter $E_T(30)$ can be adjusted to match specific target bioactives [20].

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3.3 Thermal and Chemical Stability

DES platforms demonstrate high thermal resilience up to 150 °C – 200 °C, comfortably exceeding common extraction temperatures [21]. This stability minimizes volatile loss, reduces solvent degradation, and supports energy-efficient thermal extraction [22].

4. Mechanisms of Bioactive Compound Solubilization

The enhanced solubilization performance of DESs toward diverse bioactive targets relies on cooperative supramolecular interactions [23]:

- **Hydrogen-Bonding Solvation:** Bioactives containing polar functional groups (e.g., phenolic hydroxyls in quercetin or epigallocatechin gallate) integrate directly into the solvent hydrogen-bonding framework, forming strong solute-solvent complexes [24].
- **π-π and Dispersion Interactions:** Aromatic bioactive skeletons (such as cinnamic acids, stilbenes, and coumarins) interact with aromatic or conjugated DES components through orbital overlap and dispersion forces [25].
- **Hydrophobic Entropic Driving Forces:** Type V hydrophobic DESs (e.g., thymol:menthol or decanoic acid:lidocaine) extract lipophilic targets (carotenoids, phytosterols, essential oils) via hydrophobic interactions that exclude water [12].
- **Cell Wall Deconstruction:** Acidic DES formulations act as mild digestive agents, breaking down or swelling plant cell wall polymers (pectin, cellulose, hemicellulose). This structural breakdown enhances matrix porosity and speeds intracellular solute leaching [26].

5. Advanced Extraction Intensification Technologies Integrated with DESs

5.1 Ultrasound-Assisted Extraction (DES-UAE)

Combining DES media with Ultrasound-Assisted Extraction (UAE) enhances mass transport kinetics [27]. Acoustic cavitation forms microbubbles within the liquid phase. The rapid collapse of these cavitation bubbles generates high-pressure microjets and localized shear forces that disrupt plant tissue membranes [28]. This mechanical disruption improves solvent penetration into intracellular spaces, shortening extraction durations to minutes while operating at lower temperatures to protect thermolabile bioactives [29].

5.2 Microwave-Assisted Extraction (DES-MAE)

DES media exhibit high ionic conductivity and dipole moments, facilitating rapid energy absorption under microwave irradiation [30]. Ionic conduction and dipole rotation generate fast, uniform internal heating across the mixture. This rapid temperature rise creates internal pressure within plant cells, rupturing structural membranes and releasing target compounds into the solvent phase with high selectivity [31].

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5.3 Pressurized Liquid Extraction (DES-PLE)

Pressurized liquid extraction uses elevated pressures to maintain DES media in a fluid state above their atmospheric boiling thresholds [32]. Applied pressure forces low-viscosity DES into matrix nanopores, accelerating mass transfer rates and enabling high recovery yields of bound phenolics and complex alkaloids in brief operating cycles [33].

6. Target Bioactive Classes and Application Performance

The modular solvation profiles of DES media allow for efficient recovery across primary classes of secondary metabolites, as detailed in Table 2 [18, 26, 34-36].

Target Bioactive Class	Representative Compounds	Optimal DES Formulation	Extraction Method	Key Performance Advantages	Ref.
Phenolic Acids & Flavonoids	Rutin, Quercetin, Chlorogenic Acid	Choline Chloride : Lactic Acid (1:2) + 20% H_2O	DES-UAE	30–60% yield increase over 80% ethanol; enhanced oxidative stability.	[18]
Alkaloids	Berberine, Caffeine, Piperine	Choline Chloride : Malic Acid (1:1) + 25% H_2O	DES-MAE	Rapid dissolution; high selectivity; quantitative recovery from tissue matrices.	[34]
Carotenoids & Lipids	β-Carotene, Lycopene, Astaxanthin	Thymol : Decanoic Acid (1:2) [Hydrophobic DES]	Stirred Extraction	Water-free non-polar phase; protection against photo-oxidation.	[12]
Anthocyanins	Cyanidin-3-glucoside, Delphinidin	Choline Chloride : Citric Acid (1:2) + 15% H_2O	DES-UAE	Acidic medium stabilizes the flavylium cation, preventing color loss.	[35]
Polysaccharides & Peptides	Pectin, Inulin, Bioactive Peptides	Choline Chloride : Urea (1:2) or Glycerol (1:2)	DES-PLE	Selective polymer recovery without causing hydrolytic chain cleavage.	[36]

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7. Optimization Strategies via Response Surface Methodology (RSM)

Maximizing bioactive yields while optimizing solvent usage requires multi-variable optimization strategies [37]. Response Surface Methodology (RSM), employing Box-Behnken Designs (BBD) or Central Composite Designs (CCD), evaluates process parameters and their interaction effects [38]:

1. **HBA:HBD Molar Ratio Selection:** Adjusting stoichiometry modifies hydrogen-bonding density and molecular spatial accommodation within the solvent network [15].
2. **Controlled Water Addition (0–40% v/v):** Water addition lowers dynamic viscosity and improves mass transport. However, exceeding ~35-40% water content disrupts the core HBA-HBD hydrogen-bonding network, converting the system into a simple aqueous solution and lowering extraction selectivity [18].
3. **Extraction Temperature (30 °C – 80 °C):** Elevated temperatures decrease viscosity and increase solute solubility; however, excessive thermal exposure can degrade heat-sensitive bioactives [29].
4. **Solid-to-Liquid Ratio (1:10 to 1:50 g/mL):** Lower solid-to-liquid ratios widen the concentration gradient driving mass transfer, but require larger solvent volumes during downstream recovery [37].

8. Downstream Recovery, Purification, and DES Recyclability

The non-volatile nature of DES media prevents simple distillation, requiring dedicated downstream isolation methods to recover target bioactives and recycle the solvent phase [39]:

- **Macroporous Resin Adsorption:** Resins (such as XAD-4, XAD-7HP, or AB-8) selectively bind hydrophobic bioactives from diluted DES extracts. Water washing recovers hydrophilic DES components, followed by ethanol elution to isolate purified bioactives [40].
- **Antisolvent Precipitation:** Introducing a non-solvent (e.g., water for hydrophobic DES, cold ethanol for hydrophilic DES) alters solute solubility, triggering rapid crystallization or precipitation [41].
- **Membrane Filtration:** Ultrafiltration and nanofiltration polymeric or ceramic membranes separate high-molecular-weight targets from lower-molecular-weight DES components based on size exclusion [42].
- **Supercritical CO_2 Back-Extraction:** Supercritical CO_2 selectively extracts lipophilic bioactives directly from the DES matrix without leaving toxic solvent residues [43].

Recyclability studies demonstrate that recovered DES phases can be reused across 5 to 8 consecutive extraction cycles with minimal efficiency loss (<5% reduction in total yield), supporting circular economy objectives [40, 44].

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9. Biocompatibility, Toxicity, and Ecotoxicology

Although DESs are categorized as green solvents, their toxicity profiles vary based on constituent selection, concentration, cell permeability, and target organism [45]:

- **Cytotoxicity & Membrane Permeability:** Certain quaternary ammonium salts combined with specific organic acids exhibit concentration-dependent cytotoxicity by altering cell membrane permeability [46].
- **Ecotoxicity Assessment:** Bioassays on aquatic models (e.g., microalgae, *Daphnia magna*, micro-organisms) indicate that NADESs composed of sugars, amino acids, and polyols are readily biodegradable and non-toxic, whereas metal-based or long-chain alkyl ammonium DESs require controlled waste management [45, 47].
- **In-Formula Applications:** Biocompatible NADES extracts can bypass downstream separation, allowing extract-loaded solvents to be integrated directly into topical cosmetics, oral syrups, or pharmaceutical preparations [8].

10. Techno-Economic Analysis and Industrial Scalability

Transitioning DES extraction from bench-scale research to commercial manufacturing requires addressing operational and engineering challenges, as summarized in Table 3 [44, 48].

Industrial Consideration	Technical Challenge	Engineering Solution / Future Outlook	Ref.
Raw Material Costs	High-purity HBA/HBD reagents increase operational expenditure (OpEx).	Utilize bulk agricultural-grade or upcycled bio-based HBDs (e.g., crude glycerol from biodiesel processing).	[48]
High Viscosity Pumping	Fluid transport in industrial piping requires higher energy input.	Implement inline thermal jacketing and pre-optimized hydro-dilution (15–20% water addition).	[17, 18]
Solvent Recovery Energy	Concentrating dilute aqueous washes consumes significant thermal energy.	Integrate multi-stage reverse osmosis and membrane distillation technologies.	[42, 44]
Corrosion & Equipment	Organic acid-based DESs can cause metallic corrosion in reactors.	Use high-grade 316L stainless steel, titanium alloys, or glass-lined continuous reactors.	[48]

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11. Conclusion and Future Perspectives

Deep Eutectic Solvents represent a major advancement in green extraction technology. Their unique supramolecular structure, tunable properties, atom-economical preparation, and high solvation capacity make them effective alternatives to volatile organic solvents. When paired with process intensification methods like ultrasound and microwave radiation, DES platforms achieve high extraction yields, reduced processing times, and enhanced protection for sensitive bioactives.

Future research should focus on expanding hydrophobic NADES applications for non-polar targets, refining continuous-flow extraction equipment, improving energy-efficient solvent recovery networks, and developing standardized ecotoxicological databases. Addressing these engineering and regulatory milestones will support the integration of Deep Eutectic Solvents into industrial biorefineries and green chemical manufacturing worldwide.

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