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Emerging Deep Eutectic Solvents for Sustainable Pharmaceutical Analysis: Applications, Performance, and Environmental Considerations

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Running title: Emerging DESs in Pharmaceutical Analysis

Abstract

Background and problem: Deep eutectic solvents (DESs) have become important sustainable media for pharmaceutical analysis, shifting from being just a curiosity in laboratory practice to a recognized analytical tool. The field now includes classical mixtures and tailor-made solvents with specific physicochemical properties.

Review methodology: A systematic search of relevant databases (Scopus, WoS, PubMed, and Google Scholar) was undertaken to identify studies published in peer-reviewed journals between January 2015 and June 2026. The search terms included all subclasses of DESs (NADES, HDES, MDES, SDES, SUPRADES, THEDES) and keywords related to pharmaceutical analysis. Following the initial screening of titles and abstracts, 83 manuscripts were selected for qualitative synthesis and comparative analysis. Since the selected studies differed significantly in terms of the target materials, matrices, and detection methods, a quantitative analysis or formal evidence-level assessment of the findings was not performed.

Principal findings: This review compares six DES subclasses, covering preparation, properties, and applications in sample preparation (DLLME, SPME, USAEME), chromatographic mobile phases, and stationary phases. It also evaluates sustainability-related benefits and limitations, including solvent consumption and reduction, reuse and recyclability, toxicity and biodegradability, scalability, and green analytical metrics and life-cycle assessment. Viscosity, detector compatibility, scalability, machine learning, recycling, and point-of-care miniaturization are discussed.

Conclusions: DES sustainability is not intrinsic; it depends on composition, preparation, use, recovery, and environmental evidence. Selection should balance analytical performance with environmental considerations. Trends suggest NADES suit polar analytes and biocompatibility, HDES lipophilic drugs in aqueous matrices, SDES disperser-free extraction, MDES rapid magnetic separation, SUPRADES chiral recognition, and THEDES combined delivery and analysis. A balanced selection framework is proposed.

Keywords: deep eutectic solvents; pharmaceutical analysis; microextraction; therapeutic drug monitoring; switchable solvents; chiral separation

1. Introduction

People working in the field of drug testing are well aware of the significant economic and environmental costs associated with the use of organic solvents. High-performance liquid chromatography alone uses 5-10 million liters of organic solvent per year worldwide, resulting in a significant amount of hazardous waste and a high carbon footprint [1,2]. The need to replace hazardous solvents is a pressing issue, and it has become a priority in both academia and industry [3,4]. Deep eutectic solvents (DESs) have emerged as promising low-cost, easily prepared alternatives to ionic liquids [5,6]. A typical DES can be prepared by mixing a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD) in a one-step approach, resulting in a new liquid with a melting point significantly lower than that of either of the components [7,8]. The main advantage of DESs lies in their customizability, as the choice of different HBA/HBD pairs allows for fine-tuning of polarity, viscosity, and solvation power [9,10]. The first generation of DESs consisted mainly of hydrophilic and choline-based components and was quickly followed by more elaborate systems [11]. General synthesis procedures, physicochemical properties, and applications of DESs have been reviewed elsewhere [12], as have reviews of DES-based extraction of bioactive compounds of pharmaceutical relevance [13] and the application of DESs in pharmaceutical formulation, delivery, and analysis [10,14,15].

Previous reviews have extensively covered the synthesis, physicochemical properties, and general applications of DESs but often highlight only positive aspects and lack systematic comparisons between different classes, rigorous analytical-performance evaluations, and discussion of toxicological, scalability, and sustainability evidence. Moreover, no unifying framework has been proposed for choosing a particular type of DES based on the specific needs of a given analytical task (e.g., polarity of the drug, matrix complexity, detection technique). This review focuses on six emerging subclasses of DESs that directly address some of the challenges in pharmaceutical analysis, such as isolating drugs from biological fluids, removing impurities, separating enantiomers, and even serving as drug-delivery vehicles. Unlike previous reviews, we provide a balanced overview of the strengths and limitations of each subclass and suggest a systematic, evidence-based approach to selecting among available options. Table 1 summarizes the classification discussed in this review, and Figure 1 shows the fundamental eutectic behavior, providing the rationale for the reduced melting points observed in DES systems.

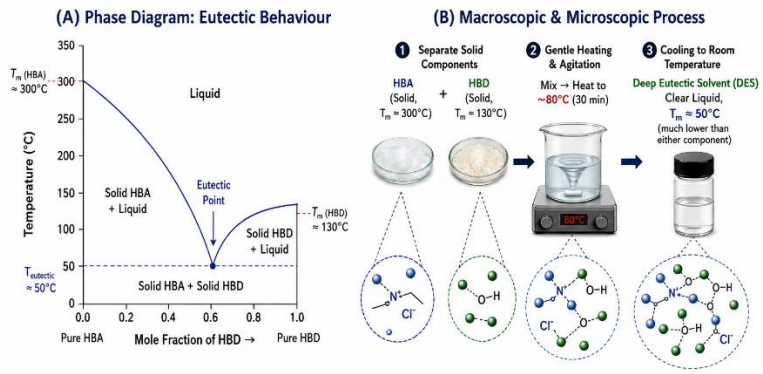


Figure 1. Schematic diagram illustrating the formation of a deep eutectic solvent (DES) from a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD). The mixture has a eutectic point, which has a significantly lower melting temperature than either of the pure components, and thus remains a clear liquid at room temperature after gentle heating and cooling.

Table 1. Classification of emerging deep eutectic solvents in pharmaceutical analysis

DES subclass	Typical HBA	Typical HBD	Key advantage	Main limitation	Ref.
NADES	Choline chloride, sugars	Citric acid, amino acids, glycerol	Biocompatibility, biodegradability	High viscosity; UV interference below 250 nm	[16,17,18]
HDES	Quaternary ammonium salts, terpenes	Fatty acids (capric, oleic), thymol, menthol, benzyl alcohol	Water immiscibility; extraction of non-polar analytes	Moderate cytotoxicity; nanoparticle dispersion issues for magnetic variants	[19–21]
MDES	Any DES + magnetic nanoparticles (Fe_3O_4, $NiFe_2O_4$)	As for parent DES	Rapid magnetic phase separation without centrifugation	Dependence on nanoparticle loading and dispersion	[22,23,24]
SDES	Fatty acids, amines	Thymol, lactic acid	Reversible switching by pH, CO_2, or temperature	Requires precise pH/temperature control	[25,26,27]
SUPRADES	Cyclodextrins (β-CD, HP-β-CD, S-β-CD)	Citric acid, lactic acid	Host–guest selectivity and chiral recognition	Requires precise pH/temperature control; high cost of macrocycles	[28,29,30]
THEDES	API (e.g., ibuprofen, lidocaine)	API or biocompatible carrier	Improved drug solubility and delivery	Characterisation of true eutectic versus solution is complex; high cost of some components	[31,32,33]

Review Methodology

A systematic literature search was conducted to ensure transparency and replicability. The databases searched were Scopus, Web of Science, PubMed, and Google Scholar. The time range for the search was between January 2015 and June 2026, with the search completed on 20 June 2026. The combined search strategy included terms related to DES subclasses ("natural deep eutectic solvents" OR "NADES" OR "hydrophobic deep eutectic solvents" OR "HDES" OR "magnetic deep eutectic solvents" OR "MDES" OR "switchable deep eutectic solvents" OR "SDES" OR "supramolecular deep eutectic solvents" OR "SUPRADES" OR "therapeutic deep eutectic solvents" OR "THEDES") and pharmaceutical analysis terms ("pharmaceutical analysis" OR "drug

extraction" OR "sample preparation" OR "microextraction" OR "DLLME" OR "SPME" OR "chromatography" OR "therapeutic drug monitoring"). The search was conducted on titles, abstracts, and keywords, and the reference lists of relevant reviews were hand-searched.

The inclusion criteria were as follows: (i) peer-reviewed original research articles or reviews; (ii) articles written in English; (iii) articles reporting quantitative analytical performance data (e.g., recovery, LOD, LOQ) or critical physicochemical characterisation relevant to pharmaceutical analysis; and (iv) articles published in the defined time window. The exclusion criteria were as follows: (i) articles focusing exclusively on non-pharmaceutical applications (e.g., catalysis, materials synthesis) without analytical relevance; (ii) conference abstracts, preprints (unless peer-reviewed equivalents were unavailable), and non-English publications; and (iii) articles that could not be retrieved in full text.

After duplicate removal, 412 records were retained and screened by title/abstract; 168 records were excluded at this stage, leaving 244 records for full-text assessment. Of these, 161 were excluded after full-text assessment, and 83 sources were retained for this review. The sources retained include original research articles, reviews, one foundational green-chemistry text, and one regulatory guidance document. The data extracted from the retained sources focused on the analyte, matrix, DES composition, extraction technique, recovery, LOD, LOQ, precision, extraction time, and reusability. Due to the differences in the experimental conditions (matrix, volume, instrument), no statistical meta-analysis was performed, and instead a qualitative comparative framework was developed to compare the strengths and limitations of each DES class. Figure 2 shows the corrected workflow of the identification, screening, eligibility, and inclusion process, including the records excluded at the title/abstract and full-text stages and the final number of included sources. No supplementary data files or auxiliary extraction tables are used; all summary data are presented in the main-text tables.

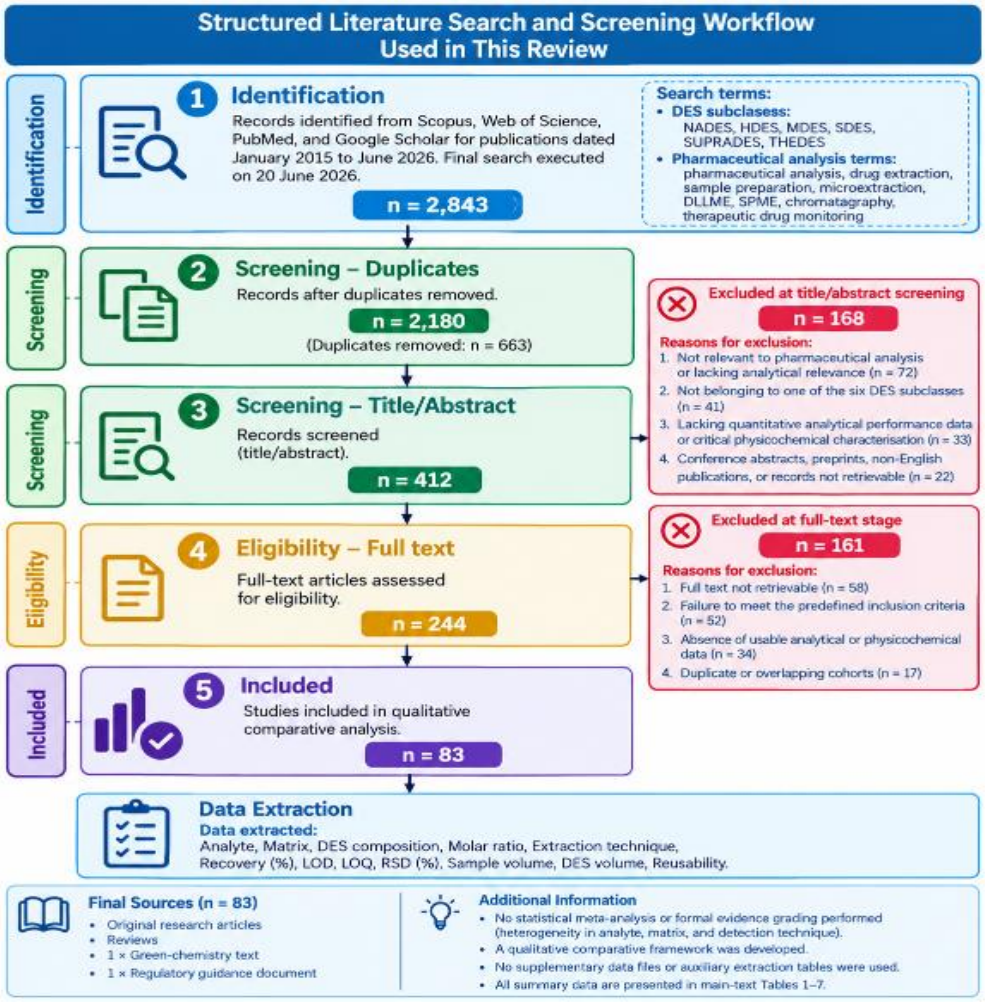


Figure 2. Corrected PRISMA-style workflow of the structured literature search and screening process used in this review. After duplicate removal, 412 records were retained and screened by title/abstract; 168 records were excluded at this stage for reasons including non-pharmaceutical/non-analytical scope, conference abstract/preprint/non-English status, and absence of quantitative analytical performance or critical physicochemical characterisation. The remaining 244 records were assessed in full text; 161 records were excluded because the full text was unavailable, the study did not report quantitative analytical or physicochemical data, the scope was non-pharmaceutical, or the inclusion criteria were otherwise not met. A total of 83 sources were included in the final review.

2. Natural Deep Eutectic Solvents (NADES)

2.1. Distinguishing Features of NADES

NADES are comprised only of primary metabolites such as sugars, amino-acids, organic-acids, polyols, and choline derivatives [16,17]. The reason is simple, solvents made from plant sources are supposed to be biocompatible and biodegradable [34]. In reality, NADES are prepared by just gently heating and stirring, often with a small amount of water (10-20%w/w) to make them less viscous [35]. From an analytical perspective, their most attractive feature is the ability to participate in multiple non-covalent interactions (hydrogen bonding, dipole forces, van der Waals attractions) with a range of analytes, from very polar alkaloids to moderately lipophilic flavonoids [36,37]. Computational tools such as COSMO-RS now allow for rapid screening of possible NADES formulations, greatly reducing trial-and-error experimentation [38,39]. Figure 3 shows the range of non-covalent

interactions NADES can form with different classes of analyte, highlighting their versatility in extraction processes.

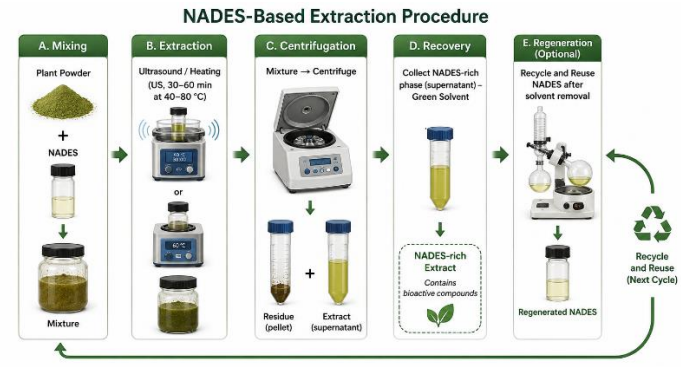


Figure 3. Schematic representation of a natural deep eutectic solvent (NADES)-based procedure for extracting bioactive compounds from plant material. Steps: (A) mixing plant powder with NADES; (B) extraction assisted by ultrasound or heating (30-60 min at 40-80°C); (C) centrifugation to separate residue; (D) recovery of the NADES-rich supernatant; (E) optional NADES regeneration and reuse after solvent removal.

2.2. Practical Applications

2.2.1. Extraction of Bioactive Substances

A number of studies have demonstrated the efficiency of NADES for the extraction of phenolics, flavonoids, and other metabolites from plant materials, as compared to methanol, ethanol, ethyl acetate, or other conventional solvents [17,40,41]. Their advantageous use is justified by both environmental sustainability and high extraction capacity. In addition, NADES are capable of extracting thermolabile compounds without damaging them with high temperatures [35], which is an important quality for the production of certain drugs or biologically active additives. The use of NADES for the recovery of biologically active substances from various types of waste materials is actively developing in line with circular economy concepts [42,43]. Among the most promising systems are choline–geranic acid (CAGE), which demonstrates exceptional permeability properties, as well as antimicrobial activity [44,45].

2.2.2. Clinical Therapeutic Drug Monitoring

NADES have also been explored in therapeutic drug monitoring (TDM). However, the menthol–thymol system, used in the detection of tyrosine kinase inhibitors (TKIs) in human plasma, as described in the original NADES literature, is classified in this review as an HDES due to the hydrophobic character of this system. Hence, this example is further discussed in Section 3.2.2 in accordance with this classification rule, which is also consistent with all other examples presented in this study. The rule is based on the water miscibility and hydrophobic/hydrophilic character of the eutectic mixture.

2.2.3. NADES as HPLC Mobile-Phase Modifiers

Attempts to use NADES as mobile-phase additives have been limited [18]. The main challenges include high viscosity (associated with increased back pressure), low volatility (incompatible with mass spectrometry detection), and high UV absorbance at wavelengths below 240–260 nm (causing interference in many pharmaceutical analyses) [46,47]. Some authors reported that a DES-based mobile-phase additive at 1.0% (v/v) improved the peak shape of basic compounds on silica-based stationary phases, and DES-based additives were reviewed in relation to chromatographic applications [47]. However, routine application is limited by the choice of available detectors [19]. Therefore, in our opinion, until these challenges are addressed (e.g., through the design of more volatile DESs or those with lower UV cut-off), their role would be mainly related to sample preparation.

2.3. Limitations Specific to NADES

The main disadvantages of using NADES in pharmaceutical analysis include high viscosity, low ultraviolet (UV) absorption below approximately 250 nm, non-volatility, and limited applicability to the analysis of non-polar analytes in aqueous media. These issues are discussed in conjunction with potential solutions in Section 9.

3. Hydrophobic Deep Eutectic Solvents (HDES)

3.1. Design and Physicochemical Basis

The conventional Designers of DESs (DESs) are hydrophilic and therefore freely miscible with water. While such properties are appropriate for most extractions, they preclude the utilization of DESs in their current form for the direct isolation of non-polar species from aqueous media. A solution to this problem was the design of hydrophobic deep eutectic solvents (HDES) through the judicious choice of water-immiscible components, such as terpenes (thymol, menthol), long chain fatty acids (capric, lauric, oleic), or quaternary ammonium salts with bulky anions [20,21]. The first report of the system was carried out by van Osch et al. in 2015, using capric acid and tetrabutylammonium chloride [21]. Since then, HDES have been widely investigated for the extraction of lipophilic species from water [19,48,49]. Figure 4 displays the most common components of such solvents, together with their characteristic features.

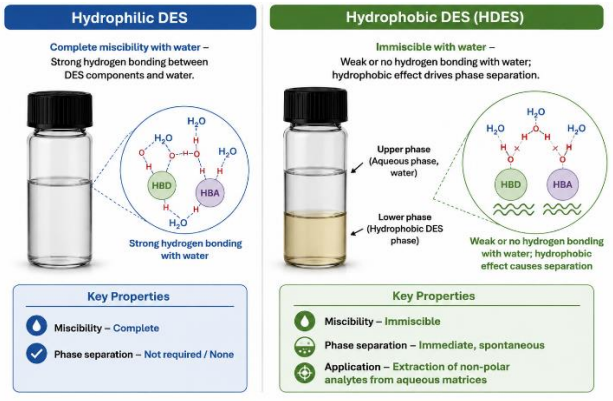


Figure 4. Comparison of a hydrophilic DES (left) and a hydrophobic DES (HDES, right) in contact with an aqueous sample. The hydrophilic DES mixes completely with water, forming a single phase. In contrast, the HDES forms a clearly separated upper or lower phase due to water immiscibility, facilitating easy isolation of the extractant without centrifugation in many cases.

3.2. Pharmaceutical Applications

3.2.1. Isolation of Drugs and Impurities

A study conducted in 2025 reported a dicarboxylic-acid-based HDES (adipic/tartaric/itaconic acid as HBD, glycerol as HBA) for the microextraction of amlodipine from tablets and pure drug solution followed by spectrophotometric determination [50]. The LOD is appropriately reported (0.71 mg/L). However, even more interesting is the study regarding the analysis of genotoxic nitrosamines in sartan drugs. Indeed, after the worldwide recall of valsartan-containing pharmaceutical products in 2018 due to the presence of N-Nitrosodimethylamine (NDMA), there was a pressing need to develop a suitable analytical method for the screening of these contaminants [51]. Hence, thymol-based HDES (thymol:benzyl alcohol) in combination with ultrasound-assisted dispersive liquid-liquid microextraction followed by HPLC-DAD was proposed by Novotny et al. [52]. The LOQs were in the range of 17–220 ng/g for NDMA and 16–290 ng/g for NMBA with satisfactory recoveries (81.8–104.2%) [52]. The method was successfully applied for the determination of nitrosamines in losartan containing dosage forms and bulk drug substances. An example of a scientific study that demonstrates the successful application of HDES for a real analytical challenge (pharmaceutical analysis of sartan drugs) is presented. Thus, Table 2 below provides an overview of the selected scientific studies along with the corresponding information about the analytical performance of the proposed approaches.

Table 2. Representative HDES-based microextraction methods for drugs and impurities

Analyte	HDES composition	Matrix	Technique	Sample vol. (mL)	DES vol. (µL)	LOD	Recovery (%)	RSD (%)	Ref.
Amlodipine	Dicarboxylic acid + glycerol	Tablets	LLME	5.0	150	710 ng/mL	91.5–107.8	<5.2	[50]
NDMA	Thymol + benzyl alcohol	Sartans	UA-DLLME	2.0	100	17–220 ng/g	81.8–104.2	<6.8	[52]
NMBA	Thymol + benzyl alcohol	Sartans	UA-DLLME	2.0	100	16–290 ng/g	81.8–104.2	<7.1	[52]

4. Magnetic Deep Eutectic Solvents (MDES)

4.1. Concept and Preparation

The traditional liquid–liquid extraction method usually involves a time- and labor-intensive process of centrifugation to remove the extractant after solvent partitioning. MDES overcomes this problem by introducing magnetic nanoparticles (Fe_3O_4, $NiFe_2O_4$, etc.) into the DES [22,53]. It is important to note that a DES can stabilise the nanoparticles and prevent their agglomeration, and the entire system is responsive to an external magnetic field [23,54]. This allows the extraction solvent to be removed from the sample matrix using a magnet within seconds [24]. Figure 5 shows the separation process using a magnet in which MDES can be isolated from complex sample matrices using an external magnet.

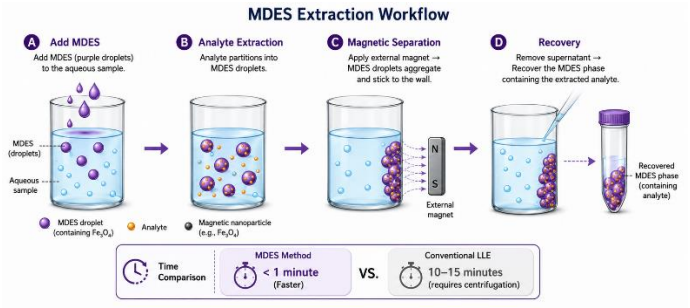


Figure 5. Workflow for magnetic deep eutectic solvent (MDES) extraction: (A) addition of MDES to the aqueous sample; (B) extraction of analyte into the MDES phase; (C) rapid magnetic separation using an external magnet (within seconds); (D) recovery of the MDES phase without the need for centrifugation. The entire process is completed in less than 1 minute, compared to 10–15 minutes for conventional liquid–liquid extraction.

4.2. Analytical Applications

4.2.1. Cephalosporin Analysis

An in-situ hydrophobic magnetic DES-based DLLME method was developed for cefixime in biological samples [55]. The magnetic separation step eliminated the need for centrifugation, and the method was coupled with HPLC for quantification. However, the authors noted that further validation with real patient samples is required, and the magnetic response was somewhat dependent on nanoparticle loading and dispersion.

4.2.2. NSAID Extraction

Another research group applied DES-modified magnetic $NiFe_2O_4$ dendrimers for the extraction of naproxen and nabumetone from pharmaceutical and biological samples before HPLC analysis [56]. The magnetic sorbents could be reused several times without considerable decrease in efficiency, which was a significant advantage over the commonly used DESs. However, it should be noted that not all of the studied MDES showed a good magnetic response; therefore, optimization of the nanoparticle amount (usually 5-15% w/w) and dispersion is required [23].

Table 3 summarizes the parameters of selected MDES reported in the literature, which allows one to compare the efficiency of the extraction and the characteristics of the methods.

Table 3. Examples of magnetic deep eutectic solvents in pharmaceutical analysis

Analyte	Magnetic Nanoparticle	DES Composition	Application	Recovery (%)	RSD (%)	Reusability (cycles)	Ref.
Cefixime	Fe_3O_4	Hydrophobic DES (thymol based)	Extraction from biological fluids	88.0-95.0	< 6.5	5	[55]
Naproxen, Nabumetone	$NiFe_2O_4$	DES-modified dendrimers	Extraction from pharmaceuticals	92.0-98.0	< 4.8	8	[56]

5. Switchable Deep Eutectic Solvents (SDES)

5.1. Operating Mechanism

SDES are "smart" solvents that are able to reversibly change their properties (usually, polarity or miscibility with water) upon the action of an external stimulus such as pH, CO_2 or temperature [25,57]. One of the classical examples is a solvent that is hydrophilic under some conditions and hydrophobic under others. Such a solvent allows one to obtain a homogeneous mixture with the sample (the rate of extraction process is increased) and separate upon trigger, without the need for a disperser solvent [58,59]. The pH-switchable SDES are especially convenient for pharmaceutical analysis. The simplest case of this design is the use of a fatty acid as one of the components: the addition of base deprotonates the acid and makes the resulting DES water-miscible, while the addition of acid back protonates it and makes the DES hydrophobic (separable from water). Figure 6 shows the switchable SDES and how their properties change upon the action of external pH or CO_2 stimulus. General reviews of DES synthesis and properties also describe switchable systems [12].

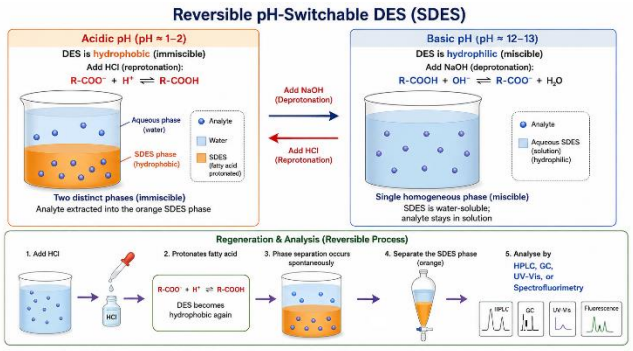


Figure 6. Reversible pH-switchable behaviour of a fatty acid/thymol switchable deep eutectic solvent (SDES). Left panel: at low pH (1-2), the fatty acid is protonated, the DES is hydrophobic and immiscible with water, forming two distinct phases. Right panel: at high pH (12-13), deprotonation renders the DES hydrophilic and water-miscible, forming a single homogeneous phase. The switching is reversible by alternate addition of acid or base, allowing easy recovery and reuse of the SDES.

5.2. Practical Examples

5.2.1. NSAIDs in Biological Fluids

A 2024 study employed a pH-switchable DES (thymol plus fatty acid) for DLLME of three NSAIDs (salicylic acid, mefenamic acid, diclofenac) from plasma, urine, and wastewater [26]. No disperser solvent was required. LODs 1–3 μg/L and recoveries 93.3–97.2% were achieved — excellent figures for therapeutic drug monitoring. The switchable nature of the DES enabled it to be easily recovered and reused for up to five cycles without a significant loss of performance.

5.2.2. Opiates in Whole Blood

The analysis of whole blood is a complicated task due to the presence of proteins and cellular debris. A promising pH-switchable DES-based LPME method has been developed for morphine and codeine [27]. The authors stated that the SDES can be regenerated by simple pH adjustment and therefore it is more economical as compared to traditional LLE. Table 4 provides an indicative comparison of SDES-DLLME and conventional DLLME for NSAID analysis; the conventional DLLME ranges are compiled from refs [59,60], whereas the SDES-DLLME values are from ref [26]. Because the studies differ in analyte, matrix, sample volume, and detection technique, this is an indicative rather than a controlled same-condition comparison.

Table 4. Comparison of switchable DES-DLLME with conventional DLLME for NSAID analysis

Parameter	Conventional DLLME	SDES-DLLME (pH-switchable)
Disperser solvent needed	Yes (e.g., acetonitrile)	No
Centrifugation needed	Yes	No (phase separation by pH change)
Extraction time (min)	10-15	5-8
Recovery (%)	80-95	93-97
RSD (%)	< 7.5	< 4.2
LOD (μg/L)	2-10	1-3

Values for conventional DLLME are indicative literature ranges compiled from refs [59,60]; SDES-DLLME values are from ref [26]. The comparison is indicative and is not a controlled same-condition comparison because the studies differ in analyte, matrix, sample volume, and detection technique.

6. Supramolecular Deep Eutectic Solvents (SUPRADES)

6.1. Principle and Chiral Recognition

SUPRADES combine the DES concept with host-guest chemistry. They incorporate macrocyclic hosts cyclodextrins (β-CD, HP-β-CD, M-β-CD, S-β-CD), calixarenes, or sulfated β-cyclodextrin into the DES network [28,61,62]. These host molecules can form inclusion complexes with guest analytes, providing a level of selectivity that ordinary DESs cannot achieve [63,64]. Binding constants can be estimated using Benesi-Hildebrand plots, and molecular modelling assists in rationalising selectivity [64]. Figure 7 illustrates the supramolecular interaction between cyclodextrin hosts and guest analytes, demonstrating the chiral recognition mechanism.

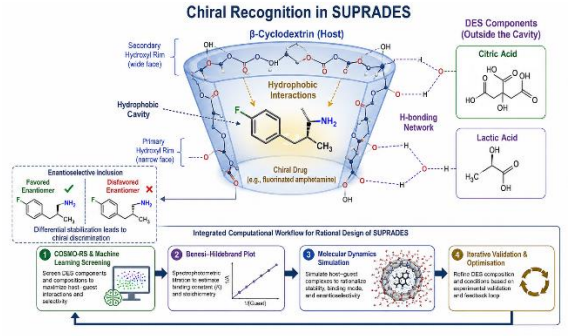


Figure 7. Supramolecular recognition mechanism in a cyclodextrin-based supramolecular deep eutectic solvent (SUPRADES). The chiral guest (e.g., a fluorinated amphetamine analog) enters the hydrophobic cavity of β-cyclodextrin. Additional hydrogen-bonding interactions with DES components (citric acid and lactic acid) enhance enantioselectivity by stabilising the inclusion complex of one enantiomer over the other. Computational tools (COSMO-RS, molecular dynamics) are used to rationalise and predict selectivity.

6.2. Applications That Count

6.2.1. Chiral Separations in CE

Stavrou and co-workers were the first to report the use of a SUPRADES (S-β-CD + citric acid) as the sole chiral selector in capillary electrophoresis for the enantiomeric separation of six fluorinated amphetamine analogs [29]. Baseline separation was achieved without any volatile organic solvent or traditional chiral additive. A follow-up study applied the same SUPRADES to nefopam and five cathinone derivatives, again providing complete separation [65]. More recently, SUPRADES composed of β-CDs and L-lactic acid demonstrated improved chiral resolution compared to single cyclodextrins or dual selector systems [30,66]. Figure 8 shows representative electropherograms demonstrating the chiral separation achieved with SUPRADES.

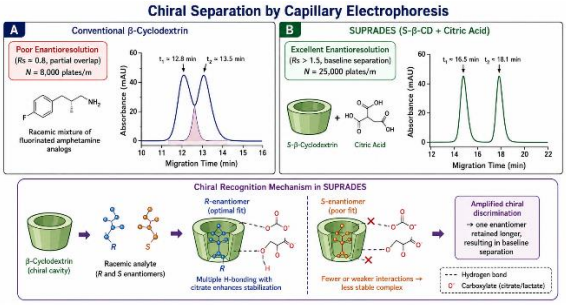


Figure 8. Representative electropherograms showing the enantiomeric separation of a racemic mixture of fluorinated amphetamine analogs. (A) Conventional capillary electrophoresis using β-cyclodextrin as the sole chiral selector: poor resolution with overlapping peaks (Rs ≈ 0.8). (B) Capillary electrophoresis using SUPRADES (S-β-CD + citric acid) as the chiral selector: baseline separation achieved (Rs > 1.5) with improved theoretical plate numbers (N = 25,000 plates/m). The longer migration times in (B) are attributed to the higher viscosity of the SUPRADES background electrolyte.

6.2.2. Antibiotic Preconcentration from Milk

A supramolecular solvent based on a hydrophobic NADES (terpenoids + carboxylic acids) and a primary amine was used to extract enrofloxacin from milk [67]. Coacervation generated mixed vesicles that efficiently captured the antibiotic. The method was straightforward, selective, and easily adaptable to other food matrices.

7. Therapeutic Deep Eutectic Solvents (THEDES)

7.1. Definition and Configurations

THEDES are DESs in which at least one component is an active pharmaceutical ingredient (API) [31,68,69]. The dual purpose is: (i) DES formation can enhance the API's solubility, permeability, or stability; (ii) the DES itself can serve as a drug delivery vehicle [32,70,71]. Several configurations exist: API as HBA, API as HBD, both as APIs, or neither component is an API alone but the eutectic mixture exhibits therapeutic activity [31,72,73]. Figure 9 presents the various configurations of THEDES, illustrating how APIs can serve as DES components. Sugar-based DESs developed for oral applications illustrate the broader biocompatibility rationale relevant to THEDES design [74].

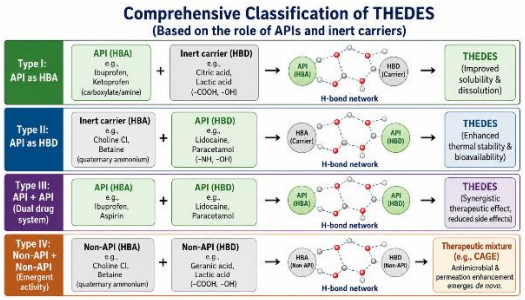


Figure 9. Comprehensive classification of therapeutic deep eutectic solvents (THEDES) based on the role of active pharmaceutical ingredients (APIs) and inert carriers in hydrogen-bonding interactions. Type I: API acts as the hydrogen-bond acceptor (HBA) with an inert carrier as HBD. Type II: API acts as the hydrogen-bond donor (HBD) with an inert carrier as HBA. Type III: both components are APIs (dual-drug system). Type IV: neither component is an API, but the eutectic mixture exhibits emergent therapeutic activity (e.g., choline-geranic acid, CAGE). Green boxes indicate APIs; grey boxes indicate inert carriers.

7.2. Relevance to Analysis

7.2.1. Physicochemical Characterisation of API-Based Systems

Several recent studies have been devoted to characterizing the thermal stability, viscosity, and surface tension of THEDESs, as a function of the molar ratio, because these are key physicochemical properties dictating their behavior in formulation and analysis [33,75]. In particular, knowledge of their phase behavior and microstructure (e.g., whether they form a true eutectic or a simple solution) is of great importance [33]. This information is mandatory to obtain reproducible analytical results with any given THEDES since each one needs to be characterized first. A representative list of THEDES properties is given in Table 5 for reference.

Table 5. Physicochemical properties of selected THEDES

API	Carrier	Molar ratio	Melting point (°C)	Viscosity (mPa·s, 25°C)	Ref.
Ibuprofen	Lidocaine	1:1	44.1	145	[32]
Lidocaine	Menthol	1:2	37.8	82	[31]
Moxifloxacin (in Ethaline)	Choline chloride + ethylene glycol	1:2	liquid at RT	36	[75]

7.2.2. Direct Spectrofluorimetric Assay

A 2026 study used Ethaline (choline chloride + ethylene glycol) as the medium for the spectrofluorimetric determination of moxifloxacin in tablets and biological samples [75]. The DES amplified fluorescence intensity, leading to lower detection limits than conventional solvents. This is a direct application of a DES (non-therapeutic in this case) as an analytical matrix, demonstrating that DES can serve roles beyond extraction.

8. Advanced Applications and Comparative Analysis

8.1. DES in HPLC Beyond Sample Preparation

There is growing interest in employing DES as mobile-phase additives. The cited study reported that a DES-based mobile-phase additive at 1.0% (v/v) improved peak symmetry for basic drugs that tail on silica-based columns [47]. DES-based stationary phases, such as deep eutectic supramolecular polymers, have also been described for HPLC separations, but they are not yet commercially available [76]. Our assessment is that these applications remain in early development and require more systematic validation against classical methods.

8.2. Synergy with Nanomaterials

The combination of DESs with nanomaterials (graphene oxide, metal–organic frameworks, etc.) allows the creation of hybrid sorbents that demonstrate significantly increased efficiency in terms of extraction. For example, the use of saffron sporopollenin modified with graphene oxide and mixed with a DES has demonstrated high performance in the extraction of acetaminophen from serum [77]. The DES was found to improve the dispersion of nanomaterial and provide additional interaction sites [53].

8.3. Comparative Performance of DES Types for a Single Analyte

Due to the different analytes, matrices, and detection techniques used at the time of the studies, it is currently impossible to compare the same analyte across different classes of DES in a controlled manner. Table 6, therefore, provides a cross-application comparison of some of the representative emerging DES-based methods that will serve to highlight their relative advantages, as opposed to providing a controlled same-analyte comparison. The data highlight various relative advantages: NADES provides the best biocompatibility but lower sensitivity for the lipophilic analytes; HDES delivers high recovery for nonpolar drugs; SDES removes the disperser and

centrifugation; MDES reduces extraction time. This framework for comparison can be extended to other analytes in subsequent research.

Table 6. Representative emerging DES-based microextraction methods for pharmaceutical analysis (including amlodipine comparison)

DES type	Analyte(s)	Matrix	Technique	Sample vol. (mL)	DES vol. (µL)	LOD	Recovery (%)	RSD (%)	Key advantage	Ref.
HDES	Nine TKIs	Human plasma	SLPME	0.5	Fibre-loaded	0.2–1.0 ng/mL	86.6–110.3	<14.1	Ready-to-use, stable, hydrophobic	[78]
HDES	Amlodipine	Tablets	LLME	5.0	150	710 ng/mL	91.5–107.8	<5.2	Simple, cost-effective	[50]
HDES	NDMA, NMBA	Sartans	UA-DLLME	2.0	100	17–220 ng/g	81.8–104.2	<7.0	Regulatory relevance	[52]
MDES	Cefixime	Biological fluids	In-situ DLLME	—	—	—	88.0–95.0	<6.5	No centrifugation	[55]
SDES	Three NSAIDs	Plasma, urine, wastewater	SDES-DLLME	5.0	120	1–3 µg/L	93.3–97.2	<4.2	No disperser, no centrifugation, reusable	[26]
SDES	Morphine, codeine	Whole blood	LPME	2.0	100	0.5 µg/L	89.0–95.0	<5.5	Clean extract, reusable	[27]
SUPRADES	Enrofloxacin	Milk	LPME (coacervation)	10.0	200	0.8 µg/L	90.0–96.0	<6.0	Selective, food matrix	[67]
THEDES	Moxifloxacin	Tablets, serum	Spectrofluorimetry	1.0	—	<0.05 µg/mL	—	<3.0	Enhanced fluorescence	[75]

9. Limitations and Unresolved Problems

It is crucial to point out that from the practitioner's perspective, there are limitations and unresolved problems associated with routine adoption. Table 7 below represents these difficulties and potential approaches to resolving them.

Table 7. Main limitations of emerging DESs and proposed solutions

Limitation	Impact	Proposed Solution	Ref.
High viscosity	Slow extraction, HPLC back-pressure	Add water (10-20%), increase temperature	[46,47]
UV absorption below 250 nm	Interference with UV detection	Use evaporative light scattering or MS after dilution	[18,47]
Non-volatility	Incompatible with GC/MS	Use as extraction solvent only, not in mobile phase	[18,47]
Lack of toxicity data	Safety concerns for pharma use	Perform OECD-based assays (in vitro/in vivo)	[34,79]
High cost of some components (e.g., cyclodextrins)	Limited scalability	Seek cheaper bio-based alternatives; develop recycling protocols	[28]
Lack of standardisation (purity, water content, storage)	Poor inter-laboratory reproducibility	Establish consensus protocols (IUPAC-style guidelines)	[11,80]

Additional considerations:

- **Toxicity:** Systematic in vitro and in vivo toxicity data remain scarce for most novel DES formulations. Reported cytotoxicity varies strongly with composition: choline-based DESs are generally less cytotoxic than some ammonium-based systems, but the available data are insufficient for quantitative ranking, and data for thymol-rich HDES remain limited [34,79]. Comprehensive profiling according to OECD guidelines is urgently needed.
- **Scalability and cost:** The preparation of samples at the laboratory scale is relatively simple; however, for all technologies presented, the transition to the pilot-industrial level is associated with significant difficulties. For example, for MDES (which requires homogeneous dispersion of nanoparticles), or SUPRADES (which requires the use of expensive cyclodextrins), this transition is not economical at the present time. The cost of components varies within a wide range. Thus, SUPRADES based on cyclodextrins are associated with the need for macrocyclic molecules, which makes them significantly more expensive than DESs based on natural sugars, choline salts, or fatty acids; this is a significant factor suppressing their development [28]. Sugar-based DESs are presented as suitable for oral administration, which highlights their low-cost and biocompatible nature [74]. Therefore, when choosing methods for obtaining DESs, researchers should be guided not so much by the unified cost characteristics as by the specific features of the selected technology.
- **Standardisation:** There is no consensus on purity criteria, water content, or storage conditions for DESs, which complicates inter-laboratory comparisons and has been noted in critical reviews of DES definition and sustainable design [11,80].

10. Future Perspectives

Machine-learning-assisted design. COSMO-RS is already a valuable tool but could be enhanced by combining it with machine learning and Quantitative Structure-Property Relationship (QSPR) models to improve the screening of new DES compositions [38,39,81]. This would allow us to move beyond trial and error and instead use predictions based on molecular descriptors to design the best DES for a given drug class.

Recyclability and closed-loop processes. SDES and MDES may offer recovery and reuse potential, although recyclability must be demonstrated for each formulation and application., and the next logical step is to integrate them into continuous extraction systems with minimal solvent loss [42,43]. This will make pharmaceutical analysis not only greener but also more cost-effective. For example, laboratory-scale recycling of SDES via pH cycling has been shown to reduce solvent consumption by more than 80% under optimised conditions in a pilot study [26], but this finding has yet to be translated to a continuous industrial setting.

Toxicological and environmental profiling. Standardised assays (e.g., OECD guidelines) should be applied at the preliminary stages to promising DESs prior to large-scale implementation. Green metrics such as EHS analysis and life-cycle assessment should become the norm [80]. We propose a tiered testing strategy as follows: (1) in silico toxicity prediction, (2) in vitro cytotoxicity (MTT assay on HepG2 or Caco-2 cells), and (3) in vivo acute toxicity (zebrafish model) for the most promising candidates.

Advanced supramolecular systems. The SUPRADES field is still in its infancy, and novel macrocycles could yield even greater selectivity for chiral drugs and isomers. Pillararenes, cucurbiturils, or modified cyclodextrins could be excellent candidates for this purpose [28,29,30]. Furthermore, coupling SUPRADES with capillary electrophoresis or micro-LC could open up new avenues for high-throughput chiral analysis.

Miniaturisation and point-of-care devices. Coupling DES extraction with microfluidics or portable sensors would be a huge step forward towards point-of-care therapeutic drug monitoring, especially in low-resource settings [82,60]. This is a long-term vision that has already begun to take shape and has several proof-of-concept studies. DESs are also being explored as green media for material preparation and may offer new immobilisation and device-fabrication routes for miniaturised analytical systems [83]. Figure 10 is a conceptual framework for the integration of DES-based extraction with portable detection systems for point-of-care applications.

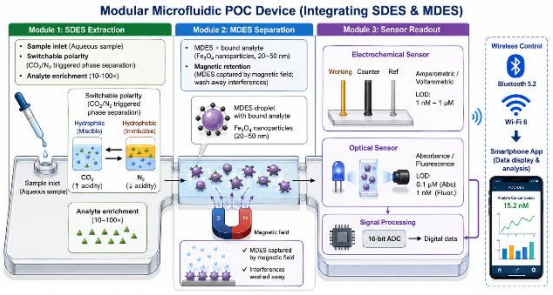


Figure 10. Conceptual design of a future point-of-care (POC) device integrating emerging DES technologies. The modular microfluidic chip comprises three main compartments: (1) switchable DES (SDES)-based extraction with CO_2/N_2-triggered polarity reversal for analyte enrichment (10-100×); (2) magnetic DES (MDES) separation using Fe_3O_4 nanoparticles (20-50 nm) for rapid phase isolation; and (3) sensor readout (electrochemical or optical) with signal processing and wireless data transmission (Bluetooth/Wi-Fi) to a smartphone application. This integrated platform aims to enable on-site therapeutic drug monitoring in resource-limited settings.

11. Conclusion

Based on the collected evidence, one can conclude that each of the six DES classes (NADES, HDES, MDES, SDES, SUPRADES, and THEDES) has its own advantages and disadvantages. Thus, NADES are the best choice for achieving high biocompatibility and maintaining natural origin. HDES are the most promising solvents for non-polar drugs' extraction from aqueous solutions. SDES change state depending on temperature and do not require disperser or centrifuge. MDES allows reducing the time of separation. SDES and SUPRADES allow making the process reversible. The use of SUPRADES is the most promising for conducting chiral analysis due to specific molecular recognition. THEDES shift the paradigm of analysis since their properties are close to ideal solutions, which allows combining delivery and analysis tasks. Currently, the most common application of DES is the preparation of samples using either HDES or SDES, but this conclusion is derived from the analysis of the papers rather than the quality assessment of the manuscripts. The risk of bias might be present due to the lack of methodological assessment. Moreover, there are still barriers for the widespread use of DES in routine laboratory practice. However, based on the collected evidence, one can suggest that with the joint efforts of various scientists, including chemists, pharmacists, modellers, and regulators, these solvents might make the pharmaceutical industry greener and more efficient in a decade. The future research should focus on designing new sustainable solvents for specific purposes with following thorough assessment of their analytical and toxicological properties.

List of Abbreviations

Abbreviation	Full Form

API	Active Pharmaceutical Ingredient
CE	Capillary Electrophoresis
CAGE	Choline-Geranic Acid
DAD	Diode Array Detector
DES	Deep Eutectic Solvent
DLLME	Dispersive Liquid-Liquid Microextraction
HBA	Hydrogen-Bond Acceptor
HBD	Hydrogen-Bond Donor
HDES	Hydrophobic Deep Eutectic Solvent
HPLC	High-Performance Liquid Chromatography
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LPME	Liquid-Phase Microextraction
MDES	Magnetic Deep Eutectic Solvent
MS	Mass Spectrometry
NADES	Natural Deep Eutectic Solvent
NDMA	N-Nitrosodimethylamine
NMBA	N-Nitrosomethylbutylamine
NSAID	Non-Steroidal Anti-Inflammatory Drug
POC	Point-of-Care
QSPR	Quantitative Structure-Property Relationship
RSD	Relative Standard Deviation
SDES	Switchable Deep Eutectic Solvent

SLPME Supported Liquid-Phase Microextraction

SPME Solid-Phase Microextraction

SUPRADES Supramolecular Deep Eutectic Solvent

THEDES Therapeutic Deep Eutectic Solvent

TKI Tyrosine Kinase Inhibitor

USAEME Ultrasound-Assisted Emulsification Microextraction

UV Ultraviolet

COSMO-RS Conductor-like Screening Model for Real Solvents

TDM Therapeutic Drug Monitoring

UA-DLLME Ultrasound-Assisted DLLME

LLME Liquid-Liquid Microextraction (my assumption, so please confirm)

GC Gas Chromatography

LC Liquid Chromatography

OECD Organisation for Economic Co-operation and Development

IUPAC International Union of Pure and Applied Chemistry

EHS Environment, Health and Safety

CD Cyclodextrin (β-CD, HP-β-CD, M-β-CD, S-β-CD)

RT Room Temperature

Rs Resolution

Consent for publication: Not applicable. This manuscript does not contain any individual person's data in any form (including individual details, images, or videos), and therefore consent for publication is not required.

Availability of data and material: Not applicable. This is a review article that synthesises existing published literature. No datasets were generated or analysed, and no supplementary data files are used. All data referenced in this manuscript are available in the cited publications.

Conflicts of Interest: The author declares no competing financial or personal interests.

Funding: This study received no external funding.

Authors' contributions: M.R. Vardast: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. The author has read and approved the final version of the manuscript.

Acknowledgements: The author gratefully acknowledges the academic support provided by the Department of Medicinal Chemistry, School of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran. The author also thanks the editorial office and reviewers for their constructive feedback.

Figures Originality Statement

All figures presented in this manuscript are original and have been created specifically for this review. They are schematic representations prepared by the author to illustrate the concepts discussed and do not reproduce any published figures. No permissions are required for their use.

AI-declaration

The author acknowledges the use of DeepSeek AI (DeepSeek-V3) as a language refinement tool to improve the clarity and readability of certain sections of this manuscript. The AI tool was employed for grammar checking, synonym suggestion, and sentence structure optimisation after the initial draft was written by the author. All scientific content, analysis, interpretation, and critical evaluation were performed by the author, who takes full responsibility for the accuracy and integrity of the work (see Section "Review Methodology" for details).

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