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Review Article

RECENT STRATEGIES FOR IMPROVING SOLUBILITY AND ORAL BIOAVAILABILITY OF PIPERINE

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ABSTRACT

Piperine, the main bioactive compound found in black pepper (*Piper nigrum* L.), has long been used in Ayurveda and traditional Chinese medicine (TCM). This compound has remarkable potential pharmacological properties, including being anti-inflammatory, antimicrobial, anticancer, anticonvulsant, antidepressant, neuroprotective, and hepatoprotective. Recent studies have reported piperine activity as an antiviral against SARS-CoV-2, which caused COVID-19. Nevertheless, the clinical use of piperine is still limited, due to its poor water solubility and bioavailability; therefore, various approaches have been developed in order to solve these limitations. This review summarises recent studies (i.e. uploaded to electronic databases in the last 10 *y*) regarding strategies that have been investigated to improve piperine's solubility and pharmacokinetic properties, using 'piperine', 'solubility', 'bioavailability', and 'formulation' as keywords. Articles that have focused on piperine as the main compound were selected and sorted based on their modification and formulation types. Studies reported various approaches: from derivatives and analogue synthesis, crystal engineering, complexation, particle size reduction (micro-and nanonisation), and lipid-and polymer-based drug delivery systems, to inorganic and hybrid nanoparticles. This review also highlights limitations and challenges for these approaches and encourages further studies to optimise piperine's potential benefits.

Keywords: Piperine, Solubility, Bioavailability, Formulations, Drug delivery systems

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INTRODUCTION

Piperine is the major bioactive component found in black pepper (*Piper nigrum* L.) that gives pepper its distinctive pungent flavor [1]. Aside from being used as a spice for culinary purposes in many countries, pepper also has a long history of use as traditional medicine. In Ayurvedic medicine, pepper is a component of 'trikatu' (black pepper, long pepper and ginger in equal proportions), which is the basis for 210 of the 370 formulations used in the Handbook of Domestic Medicine and Common Ayurvedic Remedies [2]. Pepper is also used traditionally to treat fever and various gastrointestinal conditions, as well as neurological disorders and pulmonary disorders such as asthma and chronic bronchitis [2]. Other traditional medicine systems, such as TCM, use black pepper to treat various pains (e. g. muscle aches and headaches), rheumatism, and infections such as strep throat and influenza, as well as to improve blood circulation [3]. Based on its utilisation in traditional medicine, extensive *in vitro* and *in vivo* studies regarding the bioactivity of piperine have been conducted and reported: a very wide range of bioactivity, including anti-inflammatory, antimicrobial, anticancer, anti-diabetic, anti-obesity, antihyperlipidemia, anticonvulsant, antidepressant, antiepileptic, neuroprotective, and hepatoprotective [1, 2, 4, 5]. Recent studies on piperine by *in silico* method showed that piperine has antiviral activity against the SARS-CoV-2 virus that caused COVID-19, through inhibition of virus replication and the ACE-2 receptor [6, 7].

Despite its broad and potential bioactivity, the clinical use of piperine is still limited, due to physicochemical properties that pose challenges for its development as a medicinal compound. Piperine has a low water solubility of 0.04 mg/ml which falls into the practically insoluble category based on the U. S. Pharmacopoeia, but is highly lipophilic with a log P value of 2.25 [3]. This results in dissolution as the rate-limiting step in piperine's absorption process in the gastrointestinal tract, causing an erratic oral bioavailability and low *in vivo* pharmacological effectiveness. Piperine photostability is also a problem because it is susceptible to isomerisation caused by UV rays, which also affect its concentration in preparation during manufacture, storage, and administration [8–10]. Therefore, in order to optimise piperine's therapeutic benefits and clinical applications, the problems with its physicochemical properties need to be solved by developing appropriate and effective methodological approaches, which could increase its bioavailability and pharmacological effects tremendously. This article reviews the latest developments and studies regarding strategies to improve solubility and bioavailability of piperine that have been reported in the last 10 y.

Derivatives and analogue synthesis

Synthetic approaches have been carried out to produce piperine derivatives by stereoselective fluorination, which replaces the conjugated hydrocarbon chains in piperine with vicinal difluoroalkane groups, resulting in incremental photostability after exposure to sunlight for 2.5 h [11]. This result, acrossed from intact piperine undergoing extensive decomposition in its geometric isomers, was indicated by a large number of new signals in the alkenyl region in the ^{1}H NMR spectroscopy analysis [11]. Moreover, this analogue also appears to preserve or potentiate piperine's acetylcholinesterase inhibitor activity, while simultaneously enhancing aqueous solubility [11].

Crystal engineering: polymorphism, salt, and cocrystals formation

Mostly, drugs in crystalline form have higher lattice energy, which correlates to their poor solubility in water. Utilising crystal polymorphism could lead to finding another crystalline form with better solubility. Crystal polymorphs have the same chemical composition in each crystal with different internal structures, thus improving physicochemical properties due to changes in the lattice structure and/or molecular conformation [12]. Piperine crystalline polymorph discovery has been reported and revealed in two novel polymorphs with enhanced solubility up to 1.6-fold by polymer-induced heteronucleation (PIHn) method [13]. Thermodynamic studies showed that the formed polymorph is a monotropic metastable crystalline form, which accounted for its increased solubility. Salt formation of piperine with saccharin has been reported, resulting in dissolution rate improvement [14]. The difference between pKa value of piperine (weak base pKa = 13.2) and saccharin (weak acid pKa = 1.6) allows the formation of salt-type multicomponent crystals [15, 16]. In another study, piperine cocrystal formation with succinic acid as coformer has been reported, with solubility and dissolution four and two times higher than that of intact piperine, respectively [17]. In general, improved

solubility in salt and/or cocrystal were due to decreased crystallinity and intramolecular interactions in the crystal lattice, resulting in a decrease in crystal lattice energy and its enthalpy [14, 17]. Particularly in the formation of piperine-succinic acid cocrystal, a channel motive structure is thought to be the main mechanism underlying the increase in piperine's solubility and dissolution [17].

Self-emulsifying drug delivery system (SEDDS) and micro/ nanoemulsions

Colloid delivery systems based on microemulsions or nanoemulsions are increasingly being used to encapsulate, protect, and deliver hydrophobic bioactive components. In particular, the self-emulsifying drug delivery system (SEDDS) has received considerable attention for formulating piperine in an orally administered emulsion because of its high physical stability and easy preparation [18, 19]. Piperine formulations in micro/nanoemulsion and SMEDDS/SNEDDS were successful in increasing dissolution rate and modulate pharmacokinetics, resulting in better bioavailability and bioactivity [18–20]. Modification of liquid-SMEDDS to solid-SMEDDS has also been carried out and provides a significant increment in drug release and permeation profiles–greater than liquid-SMEDDS and pure piperine [19]. Microemulsions (ME) piperine formulation, as a targeted delivery to the brain for treating Alzheimer's disease, exhibited sustained-release behaviour with superior efficacy and brain delivery compared to pure piperine [20]. In the study, tween 80 and cremophor RH40 were chosen as bioactive surfactant mixtures due to their ability to protect piperine from pre-systemic metabolism by inhibiting liver microsomal enzymes [21]. Furthermore, tween 80 has a role as a brain-targeting agent due to its ability to adsorb Apo-E from circulation and cross the blood-brain barrier by brain LDL-mediated endocytosis [22]. Nanotoxicology studies showed ME's safety in brain cells, but potential nephrotoxicity was observed and chronic use should be considered with caution [20].

Lipid-based drug delivery systems

Lipid-based drug delivery systems (LBDDS) have become foremost in recent years due to their ability to encapsulate or dissolve hydrophobic drugs in lipid, resulting in increased absorption and bioavailability. Various types of piperine formulation in lipid-based drug delivery systems have been demonstrated recently, including solid lipid nanoparticles, nanostructured lipid carriers, complexation with phospholipid, liposomes, and cubosomes.

Solid lipid nanoparticles (SLN) are the first generation of lipid-based nanocarriers which resemble emulsion structurally. The difference lies in the liquid lipids from the emulsion being replaced with solid lipids, stabilized by surfactants on the surface [23, 24]. Administration of piperine in the SLN formulation with polysorbate 80 coating (2 mg/kg) resulted in longer piperine exposure with higher levels (AUC) in the brain and demonstrated increased effectiveness on ibotenic acid-induced Alzheimer's Wistar rats, superior to Donepezil (5 mg/kg) as standard [25]. However, SLN has several drawbacks due to its highly ordered crystalline structure of solid lipids and drug expulsion during storage, including polymorphic transitions, unpredictable gelation tendencies, and low encapsulation efficiency. Therefore, the second generation of lipid carriers, nanostructured lipid carriers (NLC), was developed through the inclusion of liquid lipid in the solid lipid phase [26]. Piperine formulation with Compritol® 888 ATO as solid lipid phase and squalene as liquid lipid in NLC was recently reported as resulting in a piperine amorphous formation in NLC matrix that encapsulated efficiently (91.8% EE) with sustained-release profile (38% within 12 h) [27].

Phospholipid-drug complexation formed by hydrogen or covalent bonding can improve bioavailability by utilising the similar absorption mechanism of nutritional triglycerides and essential phospholipids through enterocytes' passive diffusion, then being transported by chylomicron into systemic circulation via the intestinal lymphatic system, bypassing the first-pass metabolism [28, 29]. Complexation of piperine with hydrogenated soya bean phosphatidylcholine (HSPC) as a phospholipid has been found to significantly increase its solubility (0.04 mg/ml to 1.18 mg/ml), dissolution efficiency and bioavailability, along with its sustained release behaviour on account of complexation by hydrogen bonding between the amide group (-NH_2) of piperine and–P=O of HSPC [30]. These results were supported by PXRD, which showed piperine-phospholipid complexation by reduced piperine crystallinity and amorphous state formation [30]. The physical mixture did not show any significant change in pharmacokinetic parameters compared to pure piperine, indicating that complexation was a determinant in pharmacokinetic improvement [30].

Liposomes are closed spherical vesicles consisting of one or more phospholipid bilayers with an entrapped aqueous phase. Liposomes could enhance bioavailability due to their good adherence to the biomembrane which increases cellular contact and diffusion across epithelial and mucosal layers [31, 32]. Liposomes also provide flexibility for a customise drug release rate that can be adjusted in accordance with the application's purpose, although thermodynamic instability is still a problem [33]. Two recent studies on liposomal piperine using soy phosphatidylcholine and dipalmitoylphosphatidylcholine (DPPC) as matrices have increased its stability at temperature exposure, indicated by a significantly reduced degradation rate along with a delayed-sustained release profile and good encapsulation efficiency, but long-term shelf stability remains a challenge [34, 35].

Cubosomes are one of the latest developments in lipid-based nanocarriers. They are thermodynamically-stable nanostructures of bicontinuous cubic phase liquid crystal, formed by certain amphiphilic lipids with self-assembly ability [36]. Their self-assembling nature leads to a relatively lower need for stabiliser/surfactant concentration compared to other nanocarriers, which is an advantage in oral delivery. Moreover, cubosomes are superior to other lipid delivery systems due to their high stability and high surface area, and thus higher loading capacity [37]. Formulation of piperine in cubosomes increased the ability to deliver piperine to the brain via the oral route for Alzheimer's therapy, due to the combined effect of Tween 80 stabilizer (brain-targeting surfactant) and poloxamer 407 (cubosomes stabilizer) with sustained-release and augmented bioactivities (cognitive function, oxidative stress parameters and AChE activity restoration to a normal level) [38]. Furthermore, nanotoxicological studies demonstrated the safety of this formulation for the liver, kidneys, and brain [38].

Inclusion complexes

Inclusion complexes are complex systems between hosts (complexing agents), which have hydrophobic (inside of cavity) and hydrophilic (outside of cavity) properties that encloses a guest molecule (mostly a hydrophobic drug). This cavity increases the solubility of hydrophobic drugs through van der Waals interactions, hydrophobic interactions, and/or hydrogen bond [39,40]. There are at least two types of inclusion complex formulations: binary inclusion complexes (BIC) which consist of piperine and a complexing agent, and ternary inclusion complexes (TIC), a supramolecular stable system which consists of a little amount of auxiliary component in a form of hydrophilic polymer in addition to BIC, and has proven its superiority in enhancing drug solubility compared to BIC [41–43]. Piperine BIC formation using cyclodextrins (CD) along with derivatives such as ethylenediamine-β-CD (EN-β-CD) and hydroxypropyl-β-CD (HP-β-CD) have succeeded in increasing solubility, dissolution rate, intestinal absorption, bioavailability, bioaccessibility, and bioactivity [42–48]. Particle size did not have a role in solubility improvement. Instead, these improvements were due to the molecular solid-state interaction between various moiety in piperine and CD, as confirmed by Raman spectra, together with a decrease in crystal lattice energy and amorphous formation as shown on PXRD analysis [46, 47]. Studies using synthetic derivatives cyclodextrins (HP-β-CD) showed a higher stability constant (Ks) and complexation efficiency (CE) than PIP/β-CD. This indicates that piperine has a greater affinity with HP-β-CD, possibly due to its better solubility and complexing properties compared to PIP-β-CD [43]. These modified cyclodextrins even have lower toxicity compared to other solubilizing agents such as surfactants and cosolvents, meaning complexation techniques have significant advantages over other delivery systems [49].

TIC formation by adding hydrophilic polymers increased Ks and CE of piperine BIC with β-CD and HP-β-CD. This would allow smaller amount usage of CD, and hence could prevent a high final dose [41–43]. One study showed the addition of d-α-tocopheryl polyethylene glycol succinate (TPGS) in piperine CD-based BIC gave synergistic action between surfactant effect of TPGS and complexation solubilisation of CD, resulting in significant enhancement of piperine's solubility, dissolution, and bioactivity [42]. Furthermore, the same study demonstrated how the microwave irradiation method produced a greater increase in solubility and dissolution compared to samples prepared by the solvent evaporation method, as uniform heat production in the sample at the same rate provides better contact between molecules [42].

Other complexing agents that have been used in inclusion complexation with piperine were kappa-carrageenan and cucurbiturils [50, 51]. Kappa-carrageenan-piperine complexation (equivalent to 100 mg/kg of piperine) showed enhancement in C_{max} and anti-inflammatory activity, even slightly higher than acetosal (45 mg/kg), compared to pure piperine [51]. In addition, cucurbiturils-piperine complexation showed a stable inclusion complex with low reactivity under the calculation of global reactivity descriptors, which could prevent piperine isomerization to chavicine [50].

Solid dispersions

Solid dispersions are eutectic mixtures or solid solutions between drugs and water-soluble carriers that increase drug solubility through amorphous formation or supersaturation, due to molecular dispersions and/or polymer solubilisation effects [31, 52]. Polymer matrices have a role in inhibiting the nucleation and recrystallisation that tend to happen due to the high energy of amorphous state and the thermodynamics or kinetics of supersaturated solution [31, 53]. Solid dispersions techniques have been demonstrated to successfully increase piperine's solubility and bioavailability by amorphous state formation which, stabilised by hydrogen bonding with Soluplus®, could hinder recrystallisation [53–56]. Eudragit L100-55 and hydroxypropylmethylcellulose acetate succinate (HPMCAS) showed acid-base interaction between their carboxyl groups and piperine, which enhanced piperine dissolution and maintained supersaturated solution [53]. Eudragit L100-55 gave the highest solubility enhancement while HPMCAS-HF gave the highest crystallisation inhibition, as its strong hydrophobic interactions with piperine and large steric hindrance obstructed diffusion-controlled crystal growth [53]. Solid dispersions have also been applied in the manufacture of fast-disintegrating tablets (FDT) from sustained-release piperine pellets (Pip-SR) consisting of piperine in solid dispersions (Pip-SD) and HPMC [56]. Pip-SR was able to maintain its structural integrity during compression, and when uniformly distributed in FDT, resulted in a similar release profile with a significant increase in bioavailability compared to both pure piperine and Pip-SD.

Polymeric nanocarriers

The greatest advantage of polymeric nanocarriers (PNCs) is their ability to be synthesized for the specific purposes and functionalities required to meet the drug's needs in controlled temporal release, as well as for targeted therapeutic applications by tailoring its properties, such as size, shape, stability, porosity, surface charge, surface chemistry, and mechanical strength. For this reason, PNCs have been the most widely used approach for piperine formulations in the past 10 y, playing a dominant role in piperine delivery, especially for cancer therapy in various forms of nanocarrier (table 1). *In vitro* studies on triple-negative breast cancer (TNBC) cells, A549 lung cancer cells, HepG2 liver cancer cells, Hs683 human brain cancer cells, HeLa cervical cancer cells, and MCF-7 breast cancer cells have shown that PNCs enhance piperine cytotoxicity, due to increased accumulation on cancer cells, higher AUC, and extended drug circulation time, along with decreased nonspecific toxicity on normal cells compared to pure piperine (table 1). Piperine/Pluronic F127 nanomicelles even showed the lowest IC50 on the Hs683 human brain cancer cells line in the study and showed a comparable result to Erlotinib as standard [57].

When designing PNCs formulations, not only the chemical compositions and backbones of the polymers matter. Polymer architectures also significantly affect the drug carrier's properties and determine its effectiveness in delivering the drug. Polymer architectures portray the shape and topology of polymers, categorised as linear, graft, branched, cross-linked, block, star-shaped, and dendron/dendrimer topology [58]. Cross-linked polymer architecture has been utilised in piperine polymer nanocarriers formulations, such as nanosponges, nanofibers mesh, and zein-κ-carrageenan nanoparticles. It has been reported that a high degree of crosslinking by diphenyl carbonate in piperine/β-cyclodextrin nanosponges resulted in the increased surface area available for drug inclusion, thus enhancing drug loading efficiency [59]. On electrospun gelatin-based piperine nanofiber mesh formulations, layer-by-layer sequential cross-linking, achieved by exposure to saturated glutaraldehyde vapour for 6-8 min to overcome gelatin's poor structural consistency in water, yielded prolonged-controlled release with minimum initial burst close to zero-order release profile [60–62]. Another study revealed κ-carrageenan hydrogel shells, formed by interfacial cross-linking using K^+ around a zein core, demonstrated cross-linker concentration-dependent particle size and drug release rate [63]. Furthermore, increased K^+concentration has been reported to yield extension of shelf-life and improved storage stability [63].

Inorganic nanoparticles

Organic nanoparticles have been developed successfully from polymers and lipids. Nevertheless, some of their inherent problems include low chemical stability, proneness to microbial contamination, and the negative effects of organic solvents used in the preparation [64]. Thus, inorganic nanoparticles that are less toxic, non-immunogenic, and highly stable compared to organic materials have been developed [64]. Hydroxyapatite is the main inorganic component of teeth and bones; thus, it has good biocompatibility compared to other inorganic materials [65]. Piperine-loaded hydroxyapatite nanoparticles, modified with phosphonate, coated with gum arabic, and conjugated to folic acid for targeted delivery systems showed a higher anticancer effect with full inhibition on monolayer HCT116 colon cancer cells and ~60% inhibition on spheroids compared to pure piperine, along with lower cytotoxicity against normal WI-38 fibroblast cells, indicating high selectivity due to conjugation to folic acid as targeting ligand through folate receptors [65]. A long-term release effect is necessary for cancer therapy; this formulation's prolonged release profile extended to>90 h, much longer than other organic delivery systems [65].

Hybrid nanoparticles (Microsphere–quantum dots)

A recent development in piperine formulation is the synthesis of nanoparticles that combine microspheres and quantum dots [66]. Microspheres based on the polymer matrix hyaluronic acid (HA)/poly (lactic-co-glycolic acid) (PLGA) coated with copper oxide quantum dots (CuQDs), as piperine delivery targeted to the brain, showed a more controlled sustained release behaviour and increased antiepileptic activity, with lower toxicity than piperine in HA/PLGA microspheres and pure piperine [66]. In targeted drug delivery systems for brain disorders, the particle size of the nanocarriers plays a significant role. CuQDs were 5 to 10 nm in size, thus facilitating effective circulation within the capillaries and an easier passage through the blood-brain fields more easily [66].

Limitations of developed formulations and future challenges

In this last decade, various formulation strategies have been developed in order to overcome piperine's problems. However, among these formulations, thermodynamic stability is still a big issue, as piperine's metastable or amorphous state formation contributes to solubility and dissolution rate enhancement, leading to uncontrolled recrystallisation during storage. Metastable polymorphs tend to change to a more thermodynamically stable form in a relatively short time, hence close monitoring of polymorphic transformations is required during their formulation, manufacture and storage [67]. In addition, polymorphs are often clinical failures once they hit the market, due to their transformation into undesirable forms that have lower solubility or are even clinically harmful [12]. Amorphous recrystallisation will bring back piperine's natural poor solubility and make the efforts made to

manage it fruitless. Thus, more studies are required of formulations' crystallisation behaviours, such as nucleation induction time, supersaturation tests as performed by [53], and shelf stability (the above-discussed studies have not done much to predict recrystallisation in storage). Solubility enhancement has been demonstrated with solubilizers such as cyclodextrins and surfactants, but studies have reported that these solubilisation mechanisms may forfeit intestinal absorption and lower apparent permeability due to reduced free drug availability for absorption [68, 69]. Therefore, further studies on formulations' effect on intestinal permeability are necessary.

Table 1: Strategies to improve solubility and bioavailability of piperine

Types and components	Techniques	Models/Methods used on studies	Result of studies	Ref
Derivatives Analogue Synthesis				
Vicinal difluoronation on C=C	Stepwise fluorination	Photostability study by sunlight exposure; solubility and bioactivity (AChE and BACE-1 inhibitor) studies	↑ Solubility and UV stability ↓ IC50 as AChE inhibitor	[11]
Crystal Engineering				
Polymorphism	Polymer-induced heteronucleation	In situ dissolution rate study by monitoring optical absorbance	↓ Thermodynamic stability ↑ Intrinsic dissolution rate 1.6-fold	[13]
Salt formation PIP-saccharin	Solvent evaporation	*In vitro* dissolution study by paddle method	↑ Dissolution rate 1.8-fold.	[14]
Cocrystal PIP-succinic acid	Solvent evaporation dan slurry	Solubility and *in vitro* dissolution study by paddle method; stability study by PXRD (one week)	↑ Aquaeous solubility 4-fold. ↑ Dissolution rate 2.2-fold. Good stability	[17]
Emulsions dan SEDDS				
SNEDDS PIP 2.5% w/w, ethyl oleate: tween 80:transcutol P	Self-emulsification	*In vitro* release study by paddle method; *in vivo* oral absorption and pharmacokinetics studies on Sprague-Dawley rats; in situ intestinal absorption study by SPIP	↑ Drug release 5.9-fold. ↑ Cmax 5-fold. ↑ AUC 5.2-fold Relative bioavailability 625,7%	[18]
SMEDDS Miglyol 812, Cremophor EL/PEG-600 and Aerosil 200 (S-SMEDDS carrier)	Self-emulsification, spray-drying	Crystalline state study by PXRD; *in vitro* anticancer study on KB cell lines by MTT assay; *in vitro* release study by paddle; ex vivo permeation study on male Wistar rats intestine; stability studies by measurement of PS, PDI, % drug content after 3 mo	S-SMDDS>L-SMDDS>pure pip Amorphous fomation ↑ Cytotoxicity in KB cells (~1.5-fold), ↑ Drug release 2-fold, intestinal permeability 1.9-fold Good stability	[19]
Microemulsion Capryol 90:Tween 80/Cremophor RH 40:Transcutol HP	Water titration	*In vitro* release study by dialysis bag method; stability studies by measurement of PS, PDI, ZP, % transmittance after 6 mo; *in vivo* studies on Alzheimer's male Wistar rats; nanotoxicological studies by clinical observations and gross organ examinations	Sustained release ↑ Bioactivities (cognitive functions, brain neural apoptosis and inflammation suppression) Potential nephrotoxicity	[20]
Solid Lipid Nanoparticles and Nanostructured Lipid Carriers				
PIP/GMS/Epikuron 200 with polysorbate-80 coating	Emulsification-solvent diffusion	Stability studies by PS, ZP, EE measurement (3 mo); *in vivo* bioactivity studies on albino Wistar rats for Alzheimer's disease	Significant changes on PS, ZP, EE (68.2 ➜ 47.5%) Amorphous state presence ↑ AUC on brain release 2.3-fold	[25]
PIP/Compritol® 888 ATO/Squalene	Solvent evaporation, high shear homogenization, sonication	*In vitro* drug release study; crystalline state study by XRD and DSC; safety profile study by haemolysis assay	Drug released 38% within 12 h, EE 91.8% Amorphous fomation No haemolysis in blood sample	[27]
Phospolipid Complexation				
PIP: HSPC	Antisolvent precipitation	Crystallline state study by PXRD; *in vitro* release studies by dialysis bag method; *in vivo* hepatoprotective activity and pharmacokinetics studies on male Wistar rats; accelerated stability studies (3 mo)	Amorphous formation, sustained release, CE 91.64% ↑ Solubility 29.5-fold, dissolution efficiency, AUC (10.4-fold), and hepatoprotective activity Good stability	[30]
Liposomes				
Nanoliposomes Piperine 2% and soya PPC: tween 80	Probe sonication	*In vitro* antioxidant potency study by DPPH radical scavenging activity; *in vitro* release kinetic study by dialysis tube diffusion; storage stability study (3 mo)	Sustained-delayed release (commenced after 2 h) ↑ Stability	[34]
Pip: DPPC	Modified reverse-phase evaporation	Stability studies (temporal, temperature, membrane release by incubation)	↑ Stability	[35]
Cubosomes				
GMO/Tween 80/Poloxamer 407	Self-assembly	*In vitro* release study by dialysis bag method; *in vivo* bioactivity and toxicological studies on male Wistar rats for Alzheimer's disease	Sustained release ↑Bioactivities	[38]
Inclusion Complexes				
TIC PIP/HP-β-CD/POLO 0,5%	Freeze drying	Phase solubility and saturation solubility studies; *in vitro* dissolution study by paddle method; *in vivo* antiinflammatory study on carrageenan-induced Wistar albino rats	↑ Solubility 13-fold, dissolution efficiency 2.8-fold ↑ Antiinflammatory, 2-fold and comparable to indomethacine)	[43]

PIP/EN-β-CD	Co-evaporation	Crystalline state study by XRD; *in vitro* dissolution study by paddle type	Amorphous fomation ↑ Dissolution 2.5-fold	[45]
PIP/α-CDs (α, β, γ)	Cogrinding (ground mixture)	Examination of crystalline state by PXRD; *in vitro* dissolution study by paddle method	↑ Solubility 17.4-fold Amorphous fomation	[46,47]
TIC (HP β CD and TPGS)	Solvent evaporation and microwave irradiation (comparative)	Phase solubility and saturation solubility study; *in vitro* dissolution study by paddle method; *in vitro* antioxidant and antimicrobial assay	MI>SE, amorphous formation ↑ Solubility 52.7-fold, dissolution 5.5-fold ↑ Bioactivities (Antioxidant and antimicroba)	[42]
PIP/β-CD	Freeze drying	*In vitro* bioaccessibility, pungency, antioxidant capacity by simulated gastrointestinal digestion and colonic fermentation	↑ Bioaccessibility, intestine permeability, antioxidant capacity Not effective in masking pungency	[48]
TIC (β CD and HPMC)	Solvent evaporation and microwave irradiation	Crystalline state studies by SEM and XRD; phase solubility and saturation solubility studies; *in vitro* dissolution study by paddle method; *in vitro* antioxidant and antimicrobial assay	MI>SE, Ks 464 M^{-1}CE 6.6 Amorphous formation, ↑ solubility 52.7-fold, ↑ dissolution 4.4-fold ↑ Bioactivities	[41]
PIP/Cucurbiturils	Not stated	Molecular interaction and stability studies	↑ Stability	[50]
PIP/κ-carraageenan	Ground mixture	*In vivo* studies on Wistar rats	↑ Cmax 2.8-fold, ↑ anti-inflammatory activities	[51]
Solid Dispersions				
PIP/HPMCAS and PIP/Eudragit L100-55	Solvent evaporation	Crystallization behavior studies; *in vitro* dissolution study under non-sink condition; in situ SPIP studies on male albino Sprague-Dawley rats	↑ Recrystallization inhibition ↑ Solubility, drug release, permeability	[53]
PIP/Soluplus®	Hot melt extrusion	*In vitro* release study by paddle method; ex vivo permeablity study by non-everted intestinal sacs of male Spargue-Dawley rats	Amorphous fomation, ↑ Solubility 160-fold, ↑ absorption 122-fold	[54]
PIP/PVP PIP/PEG PIP/Sorbitol	Solvent method	Crystalline state studies by SEM and HR-XRD; *in vitro* dissolution profile study by paddle method	Amorphous fomation, ↑ Dissolution (20-fold by PVP, 17-fold by PEG, 16-fold by sorbitol)	[55]
FDT PIP: PVPK25:phospolipid with HPMC-K100	Solvent (SD); extrusion-spheronization (pellet); wet granulation (FDT)	Crystalline state studies by SEM and XRD; *in vitro* dissolution study by rotary basket method; *in vivo* pharmacokinetics study on male beagle dogs; accelerated stability study (4 w)	Amorphous fomation, ↑ Solubility 4.5-fold (SD), sustained release ↑ t1/2 1.5-fold, MRT 1.4-fold, Cmax 2-fold, AUC 2,7-fold, relative bioavailability 267,7% Relatively hygroscopic with PIP content reduction 3.91%	[56]
Polymeric Nanocarriers				
Nanosuspensions *P. nigrum* extract and HPMC	Antisolvent precipitation	In vtiro dissolution study by paddle method, *in vivo* pharmacokinetics study on male Wistar albino rats	↑ Dissolution 3.65-fold, Cmax 1.73-fold, bioavailability 2.7-fold, and MRT	[84]
PIP/Pluronic F127 Nanomicelles with Trimethyl-chitosan coating	Nanoprecipitation-liophilization	*In vitro* release study; *in vitro* cytotoxicity studies on human brain cancer cells Hs683	Sustained release, ↑ Cytotoxicity with decreased IC50	[57]
PIP/Soluplus®/TPGS Nanomicelles	Thin-film hydration	*In vitro* release study by dialysis method; *in vitro* cytotoxicity studies by MTT assay and intracellular uptake of micelles on A549 lung cancer cells and $HepG_2$ liver cancer cells; *in vivo* pharmacokinetics study on male Sprague-Dawley rats	Sustained-release, EE 90.9%, DLC 4.67%, ↑ AUC 2.56-fold and MRT 1.2-fold ↑ Antitumor efficacy	[85]
PIP/Gum resin Nanocapsules	Emulsion diffusion	*In vitro* release study by dialysis bag method; *in vitro* evaluation of growth inhibition efficacy against *T. evansi*	Sustained release, ↓ IC50 3-fold	[86]
PIP/Starch Nanoparticles	In situ nanoprecipitation	*In vitro* release study by sonication	↑ Solubility, controlled release, DLC 4.74 mg/mg	[87]
PIP/Aptamer/PEG/PLGA Nanoparticles	Single emulsification, solvent evaporation	*In vitro* studies on MCF-7 cells	Sustained-release, ↑ Circulation time	[88]
Human Serum Albumin Nanoparticles	Self-assembly, desolvation (comparative)	*In vitro* release by dialysis bag; *in vitro* cytotoxicity studies on MCF-7 cells by MTT assay	Self-assembly>desolvation EE 76.8%, LC 8.92% ↑ Drug release, cytotoxicity, circulation time, accumulation on cancer cells	[89]
Core-shell PIP-CoQ10/Zein-κ-Carraageenan Nanoparticles	Antisolvent precipitation, stepwise electrostatic deposition	Stability studies (physical, thermal, 4-weeks storage, and photostability); crystalline state study by XRD; *in vitro* gastrointestinal digestion	Controlled release, ↑ Stability (half-live 1.8-fold, retention rates on thermal treatment 200% and 131% on storage), amorphous formation	[63]
Multilayer PIP-CoQ10/Zein-Pectin-Chitosan Nanoparticles	Antisolvent precipitation, stepwise	Stability studies (physical, thermal, storage, and photostability); crystalline state study by XRD; simulated digestion evaluation	Amorphous formation, ↑ Stability	[90]

	electrostatic deposition			
PIP/Eudragit S100 Nanoparticles	Nanoprecipitation	*In vitro* release study by dialysis bag method; *in vivo* pharmacokinetics characteristics studies on Sprague-Dawley rats; *in vivo* antiepileptic studies on zebrafish and male Kunming mice	↑ Dissolution rate, cumulative release 3-fold, bioavailability 2.7-fold, brain concentration 16-fold ↑ Antiepileptic effects	[91]
PIP/pCA-HT-chitosan mucoadhessive nanoparticles	Electrospray ionization	*In vitro* release by dialysis bag (SIF pH 1.2, SCF pH 4.0, SIF 6.4, 100 rpm)	Sustained release	[92]
PIP/HP-starch nanoparticles	In situ nanoprecipitation	*In vitro* release study in PBS (pH 1.2, 7.4, 8.6) at 37 °C	Controlled release	[93]
PIP/mPEG-PLGA nanoparticles	Thin-film hydration	*In vitro* studies on TNBC cells by MTT assay	↑ Selectivity towards non-cancer cells	[94]
PIP/poli(ε-caprolactone)/gelatin nanofibrous patches	Electrospinning	*In vitro* release kinetics study; *in vitro* study on HeLa and MCF-7 cancer cells and non-cancerous cells (NIH3T3 and human mesenchymal stem cells)	Sustained release ↑ Selectivity towards non-cancer cells	[95]
PIP/Gelatin/GTA nanofiber mesh	Electrospinning Sequential crosslink	*In vitro* degradation and swelling study; thermal stability studies; *in vitro* drug release study	↑ Nanofiber structure integrity ↑ Stability (compared to one time direct crosslinked fiber mesh) Sustained release	[60]
PIP/Gelatin/GTA nanofiber mesh	Electrospinning, multilayer-sequential crosslink	*In vitro* degradation study; thermal stability studies; *in vitro* drug release study	No significant changes compared to nonmultilayer PIP/gelatin/GTA nanofibers	[61,62]
PIP/β-CD/DPC nanosponges	Solvent	Crystalline state study by XRD; drug release study was not studied	NS in *para*-crystalline structure	[96]
PIP/β-CD/DPC nanosponges	Microwave-Assisted Fusion	Crystalline state study by PXRD; drug release study was not studied	NS in crystalline structure Crosslinking increase NS crystallinity and LE	[59]
Inorganic Nanoparticles				
PIP/Hydroxyapatite-phosphonate NPs functionalized with gum arabic/folic acid	Hydrothermal	*In vitro* release studies by bottle method with cellulose dialysis bag; *in vitro* study on HCT116, Caco2 colon cancer cells and MCF-7 breast cancer cells	↑ Release rate at pH 5 and 6.8, prolonged release at pH 7.4 ↑ Inhibitory effects toward cancer cells (full inhibition on HCT116) ↓ Toxicity on normal cells	[65]
Hybrid Nanoparticles				
Hyaluronic acid/PLGA+CuO Quantum Dots PIP Microspheres	Precipitation, emulsification-solvent evaporation	Crystalline state study by XRD; *in vitro* release study by biolysis bag protocol; MTT assay and *in vivo* antiepileptic activity by kindling procedures on male Wistar rats	Controlled sustained release ↓Toxicity, ↑ Selectivity, ↑ Antiepileptic and anticonvulsant	[66]

Polymer-based formulations have limitations. Many polymer carriers are hygroscopic (as shown on piperine solid dispersions fast disintegrating tablets) and cause system plasticisation, increasing the mobility of the API molecules to crystallize [70]. In addition, drug formulations have high doses due to the final product's increased volume [71]. Piperine lipid-based formulations still need to be improved for long-term storage stability. A potential strategy that may possible to resolve the stability problem is pro-form formulations (proliposomes and procubosomes). Proliposomes and procubosomes are dry and free-flowing powders that could form liposomes and cubosomes by hydration with water, either through contact with physiological fluid or reconstitution right before administration, and thus can be stored for long time [72–74]. Proliposomes has proven able to improve drug compounds with poor solubility and bioavailability significantly in several studies [73–76]. However, there has been only one study on procubosome formulation to date [72]. Procubosome compressed into tablet form successfully improved the solubility of BCS class II drug clopidogrel and even obtained superior C_{max}, T_{max}, bioavailability, and antiplatelet activity, compared to commercial Plavix® as standard. Moreover, procubosome formulation has successfully retained the fresh tablet drug content (98.5%) after storage for six months [72]. Therefore, this strategy could lead to an interesting future development of high stable lipid-based drug delivery systems that enhance piperine solubility and bioavailability, as well as those of other drug candidate compounds facing the same limitations.

Concerning to the number of uses of nano-sized drug delivery systems in piperine formulations, toxicity must be investigated carefully since the materials used in nanoformulation have different properties depending on size, resulting in increased tissue and organ uptake [20, 77]. Previous studies have demonstrated the possible toxicity of nanosystems and nanosurfactants and their ability to interact differently with biological and cellular barriers [20, 78, 79]. Taking these studies into consideration, it is important to reassess safe concentrations of the nanomaterials and conduct more nanotoxicology studies. In addition, any modifications to nanoparticles as carriers have to be evaluated thoroughly, because immune system responses could reduce their half-life [80]. Study of formulations' UV stability is still lacking. This property is actually really important for consideration since UV can directly diminish piperine level through photoisomerisation. Derivatives and analogue synthesis derived from piperine studies with solubility and bioavailability enhancement as objectives are also still limited, despite the considerable number of piperine derivatisation studies, as well as studies that have used this approach to solve similar problems on BCS class II drug compounds. This approach also may lead to the finding of a soluble prodrug derived from piperine–actually an older but time-tested strategy to overcome the solubility problem, and which can even be used as targeted drug delivery in the more novel application of this strategy [81–83].

CONCLUSION

Piperine has gained interest as a phytocompound that displays multiple great beneficial effects as a therapeutic agent. However, its poor solubility and bioavailability limit its biological effects *in vivo* and thus its clinical applications. In the last decade, various formulation strategies with different methodological approaches have been performed, improving piperine's physicochemical, pharmacokinetic, and pharmacodynamics properties. An increase in bioactivity, particularly on brain diseases and cancers, has been demonstrated as the outcome of these approaches. These results encourage us to perform further stability and *in vivo* studies,

continuing these efforts to optimise piperine's potential so it can be used for clinical applications in the future.

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AUTHORS CONTRIBUTIONS

Design and conception of this study were led by Erizal Zaini. All authors contributed substantially in article drafting or critical revisions for important intellectual content and give final approval of the version to be published.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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