Journal Syifa Sciences and Clinical Research (JSSCR)
Volume 8 Number 1, 2026
Journal Homepage: http://ejurnal.unq.ac.id/index.php/jsscr, E-ISSN: 2656-9612 P-ISSN:2656-8187
DOI: https://doi.org/10.37311/jsscr.v8i1.37090

Warfarin Nanoparticle and Microparticle Technologies: Therapeutic and Analytical Perspectives

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ABSTRACT

Warfarin is an oral anticoagulant widely used for the prevention and treatment of thromboembolic disorders. However, its clinical use is limited by a narrow therapeutic index, high variability in plasma levels, and the need for strict therapeutic monitoring. To address these limitations, various drug delivery systems have been developed to improve warfarin bioavailability, stabilize plasma drug concentrations, and enhance patient compliance. This review evaluates 11 representative studies covering diverse nano- and microtechnology-based approaches developed to address these challenges from both therapeutic and analytical perspectives. Therapeutic strategies include polymeric micro- and nanoparticles, self-assembled systems, silica-based carriers, and nanoprecipitation techniques, which have been shown to improve warfarin solubility, encapsulation efficiency, release control, and biodistribution, primarily in in vitro and animal models. In addition, analytical advances such as nanomaterial-based electrochemical sensors and mesoporous silica adsorbents have demonstrated enhanced sensitivity and faster detection of warfarin, with several platforms achieving limits of detection within clinically relevant ranges and validation in serum or blood matrices. Despite these promising developments, most reported outcomes remain descriptive and preclinical, with limited evaluation of long-term safety and clinically relevant haemostasis endpoints. Future efforts should focus on scalable manufacturing, comprehensive in vivo assessment of haemostasis parameters, and validation of analytical platforms using patient samples in comparison with gold-standard analytical methods. These steps are essential to enable the safe and effective clinical translation of nano- and microtechnologies for warfarin.

Keywords:
Warfarin; Nanoparticles; Microparticles; Warfarin Development; Warfarin Delivery System

Received:	*Accepted:*	*Online:*
2026-01-23	*2026-02-24*	*2026-03-01*

1. Introduction

Warfarin was first developed by Prof. Karl Paul Link in 1939 following the isolation of dicoumarol, a compound found to inhibit blood coagulation, and was later refined into a more potent derivative named warfarin [1],[2]. Initially used as a rodenticide, its clinical anticoagulant potential was revealed after a documented human overdose case demonstrated reversible hypoprothrombinemia, leading to subsequent animal studies and early clinical trials [1],[2],[3]. To overcome its poor water solubility, warfarin was later formulated as warfarin sodium, and since its introduction into

medical practice in the 1950s, it has remained a cornerstone therapy for the long-term prevention and treatment of thromboembolic diseases [1],[2],[3].

Warfarin is a therapeutic option for the treatment and prevention of thrombosis in various medical conditions [4],[5]. Warfarin remains the first-line therapy compared with direct oral anticoagulants (DOACs), such as rivaroxaban, apixaban, edoxaban, and dabigatran, due to its relatively low cost, lower risk of gastrointestinal bleeding, and lower mortality rate [4]. In addition, warfarin is superior in patients with antiphospholipid syndrome, valvular atrial fibrillation, and especially those with mechanical heart valves [4],[6]. Although the use of warfarin has been well established and it is still widely prescribed, warfarin has a narrow therapeutic window. Its bioavailability can be affected by variability in gastrointestinal absorption and hepatic metabolism, and warfarin may form a poorly water-soluble form when exposed to the acidic pH of the stomach. Beyond these pharmacological limitations, other challenges include low patient adherence due to complex dosing regimens, the need for routine and careful INR monitoring, and dietary and concomitant medication restrictions because warfarin interacts with many foods and drugs. These factors make it difficult for patients to adhere consistently to therapy, thereby increasing the risk of adverse effects and therapeutic failure [3],[7],[8],[9]. These limitations have driven extensive research into the development of warfarin-related technologies to ensure effective and safe use, including controlled drug release systems, protection of warfarin from direct interactions (with food, drugs, or non-target sites) within the body, and monitoring of warfarin distribution. Such approaches aim to minimize adverse effects, enable accurate drug-level monitoring, improve patient convenience, and enhance therapeutic efficiency [10],[11],[12].

Recent studies indicate that conventional approaches are limited, while chromatographic methods such as HPLC or LC–MS/MS for warfarin analysis, although sensitive and accurate, are costly, time-consuming, and require complex sample preparation. Therefore, nanotechnology- and microtechnology-based methods have been developed as advanced drug delivery systems to overcome various drug delivery challenges, such as achieving targeted delivery, protecting drugs from premature degradation, enhancing bioavailability, and preventing direct contact with non-target organs, thereby reducing the likelihood of adverse effects [10],[11],[12]. In addition to their role as drug delivery systems, nanoparticles can also function as carrier markers or electrochemical sensors for detecting drug distribution, enabling monitoring of drug release while improving sensitivity, selectivity, and detection speed [13],[14].

This review integrates advances in nano-/micro-formulated warfarin delivery with analytical and sensor-based monitoring to address safety, release control, and therapeutic drug-monitoring challenges, and to highlight emerging applications beyond anticoagulation.

2. Methods

Study design

This article was prepared as a structured literature review to synthesise therapeutic nanoparticle/microparticle delivery platforms and analytical technologies relevant to warfarin.

Search strategy and information sources

A comprehensive literature search was conducted in Elsevier, PubMed Central (PMC), and Google Scholar to identify studies on warfarin nanoparticle/microparticle technologies and warfarin-related analytical platforms. Searches covered August 1953

to January 2026, with the last search performed on 21 January 2026. The search used the following keyword combinations: (1) "nanoparticle" AND ("warfarin" OR "warfarin sodium"), (2) "microparticle" AND ("warfarin" OR "warfarin sodium"), and (3) "warfarin" AND ("delivery system" OR "warfarin sodium"). Reference lists of eligible papers were also screened to identify additional relevant records.

Eligibility criteria

Studies were included if they were peer-reviewed primary articles that (1) investigated warfarin or warfarin sodium formulated as nano/microparticles or evaluated warfarin-relevant adsorption, sol–gel, or sensor platforms, and (2) reported extractable performance outcomes such as particle size and morphology, polydispersity index or zeta potential, encapsulation efficiency or drug loading, release behaviour or release kinetics, pharmacokinetics or biodistribution, haemostasis-related outcomes (including PT/INR where available), or analytical performance metrics (LOD/LOQ, linear range, and recovery in relevant matrices). Studies were excluded if they were duplicates, non-original publications (reviews, editorials, letters, or conference abstracts), not related to warfarin formulation or analytical-platform development, written in Indonesian, or did not report sufficient characterisation and evaluation data.

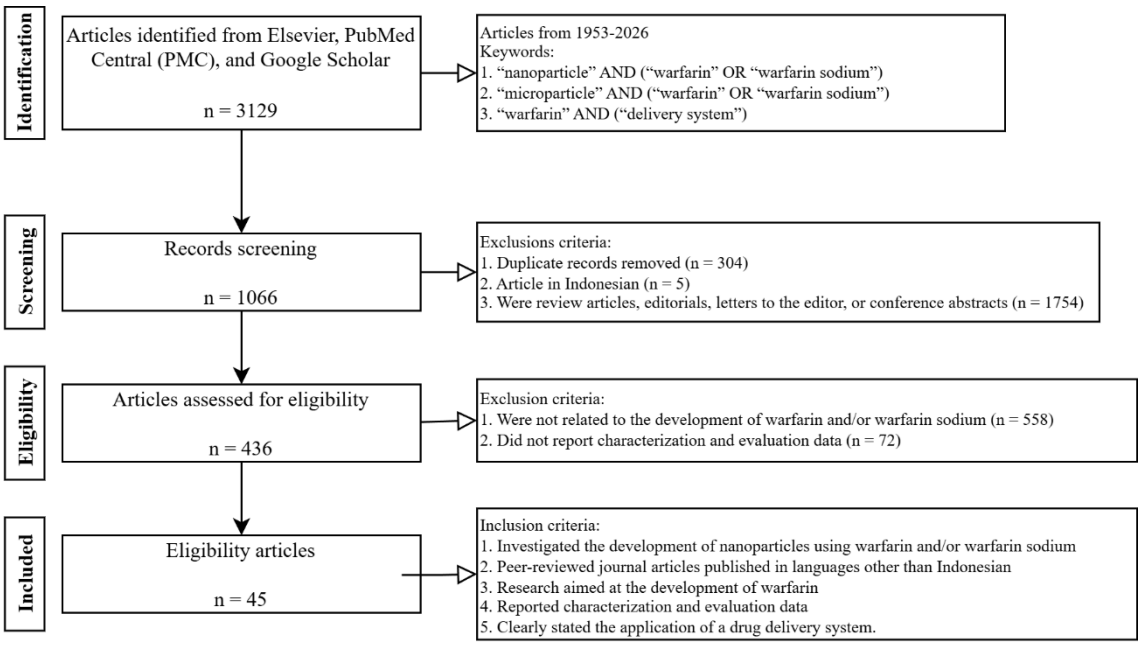


Figure 1. PRISMA flow diagram of the literature search, screening, and study selection process (1953–2026).

Study selection and data extraction

Records were screened by title and abstract followed by full-text eligibility assessment. The selection process is summarised in **Figure 1**. A total of 3,129 records were identified. After removing duplicates (n = 304), excluding Indonesian-language records (n = 3), and excluding non-original publications including reviews, editorials, letters, and conference abstracts (n = 1,754), 1,066 records remained for screening. During screening, 558 records were excluded because they were not related to warfarin or warfarin sodium development, and 72 records were excluded because they did not report characterisation or evaluation data, leaving 436 full texts assessed for eligibility.

Finally, 45 eligible articles were included in the synthesis. Data were extracted into a structured matrix capturing study context (platform type, materials, preparation method), key performance outputs (size, encapsulation or loading, release behaviour, pharmacokinetic or analytical metrics), and translational considerations (model system, limitations, and clinical readiness).

Synthesis approach

Evidence was synthesised narratively and grouped into two domains: (1) therapeutic delivery platforms (polymeric micro/nanoparticles, self-assembled systems, silica-based carriers, nanoprecipitation, and sol–gel-derived matrices), and (2) analytical and monitoring technologies (electrochemical sensors, voltammetric platforms, and adsorption-based approaches). Findings were interpreted with emphasis on comparability of outcome measures and translational constraints relevant to warfarin's narrow therapeutic index.

3. Results and Discussion

Warfarin Mechanism of Action

Warfarin is an anticoagulant drug that produces its anticoagulant effect through inhibition of vitamin K epoxide reductase, a key enzyme involved in vitamin K recycling, this inhibition limits the availability of biologically active vitamin K, resulting in reduced activation of vitamin K–dependent coagulation factors (II, VII, IX, and X) and the anticoagulant proteins C and S [15], [16]. Anticoagulant therapy is intended to limit the progression of established thrombi and to reduce the risk of subsequent thromboembolic events that could lead to severe or life-threatening consequences [16]. Vitamin K is essential for the γ-carboxylation process of coagulation factors II, VII, IX, and X, a modification required to enable their functional activity in the blood clotting cascade. This process enables these factors to bind calcium ions and interact with platelets during the coagulation cascade. In the absence of γ-carboxylation, these coagulation factors remain inactive, thereby disrupting coagulation and preventing clot formation [15], [17]. In addition, the anticoagulant proteins C and S also require γ-carboxylation to become biologically active. Consequently, inhibition of vitamin K epoxide reductase by warfarin disrupts the vitamin K recycling pathway, thereby preventing the reduction of vitamin K epoxide into its biologically active form, vitamin K_1 [17].

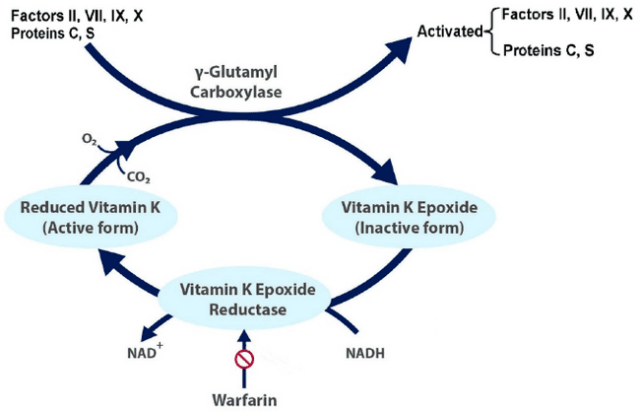


Figure 1. Warfarin mechanism of action

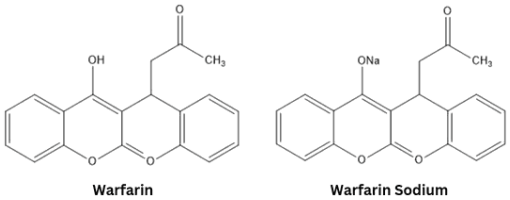
Warfarin sodium is a derivative or salt form of warfarin that exhibits approximately 75,000-fold higher water solubility than native warfarin, while maintaining effective anticoagulant activity in reducing blood coagulation. This formulation is particularly advantageous when administered intravenously, as it avoids variability associated with gastrointestinal absorption and thus provides a more consistent therapeutic response [3].

To provide mechanistic context for these formulation and monitoring challenges, **Figure 2** illustrates warfarin's mechanism of action through inhibition of vitamin K epoxide reductase, resulting in reduced γ-carboxylation and functional activation of vitamin K–dependent clotting factors.

Characteristics of Warfarin Sodium

Table 1 summarises key physicochemical and clinical characteristics of warfarin sodium that motivate advanced formulation and monitoring strategies, including its narrow therapeutic index, variability in response, extensive drug–drug and food interactions, and the need for careful dose titration.

Table 1. Characteristics of warfarin sodium [18], [19], [20]

Parameter	Spesification
Molecular Formula	$C_{19}H_{15}NaO_4$
Molecular Weight	330,31
Molecular Structure	 Warfarin Warfarin Sodium
Description	Amorphous form or crystalline powder; white in color; odorless; slightly bitter taste. Loses color upon exposure to light
Solubility	Very soluble in water; freely soluble in ethanol; very slightly soluble in chloroform and ether
pH	Between 7,2 and 8,3
Water Content	(Method I): Not more than 4.5% for the amorphous form; and not more than 0.3% for the clathrate crystalline form
Organic Impurities	Each individual impurity not more than 0.3%, and total impurities not more than 1.0%
Indication	According to FDA labelling: 1. Prophylaxis and treatment of venous thrombosis and pulmonary embolism 2. Prevention and management of thromboembolic disorders related to atrial fibrillation or prosthetic heart valve implantation 3. Reduction of the risk of death, recurrent myocardial infarction, and thromboembolic events following myocardial infarction. Off-label: Secondary prevention for stroke recurrence and transient ischemic attacks (TIA)
Packaging and Storage	Stored in tightly closed, light-resistant containers

Development of Warfarin/Warfarin Sodium

Warfarin is a vitamin K antagonist anticoagulant whose clinical benefits have been well established and that remains in use to this day. However, warfarin is classified as a drug with a narrow therapeutic index, making its use challenging because it requires

frequent and careful monitoring. These limitations have driven extensive efforts to further develop warfarin in order to improve its ease of use, safety, and therapeutic effectiveness [10], [11], [12]. Several studies reviewed demonstrate that advances in nano- and microtechnology have yielded significant results in overcoming the limitations of warfarin, from both therapeutic and analytical perspectives. These approaches have been shown to enhance sensitivity, selectivity, and control over warfarin interactions within the biological environment, which has long been a major challenge in the management of drugs with a narrow therapeutic index.

Therapeutic Delivery Platforms

a.) Methods

Oil-in-Water (o/w) Emulsion

The study by Scala-Bertola et al. (2012) aimed to assess the impact of warfarin formulation and subcutaneous delivery on its pharmacokinetic characteristics, particularly slower absorption and more stable drug exposure without reducing bioavailability. This approach was motivated by the narrow therapeutic index of warfarin and its high plasma concentration fluctuations, which increase the risk of bleeding. The study employed a simple oil-in-water (o/w) emulsion method and successfully produced warfarin microparticles using poly(ε-caprolactone) (PCL) as the base material. Pharmacokinetic evaluation involved measuring plasma warfarin concentrations at various time intervals after administration, followed by the calculation of critical parameters such as C_max, T_max, AUC, and half-life. The results showed that subcutaneous administration of PCL–warfarin without PVP produced particles with a mean size of 67 ± 6 µm, an encapsulation efficiency of 47.4%, and controlled release for up to 72 hours. In contrast, PCL–warfarin formulations containing PVP produced particles of 72 ± 1 µm with a lower encapsulation efficiency (37.3%) and a faster release rate, with approximately 80% of the drug released within 72 hours, due to increased particle porosity induced by PVP. Furthermore, the formulations exhibited lower C_max and longer T_max values than conventional warfarin preparations (solution and suspension), while maintaining adequate AUC. However, when the biological effects were compared, the microparticle formulations, solution, and suspension showed no significant differences in prothrombin time (PT), indicating that the microparticles still had limitations in achieving long-term plasma concentration stability. Overall, this study demonstrated that subcutaneous warfarin formulations could improve the pharmacokinetic profile by providing more stable plasma levels and controlled absorption, although further evaluation remains necessary [21].

Ionotropic gelation

Similar to Scala-Bertola et al. (2012), Khalil et al. (2012) developed warfarin nanoparticles based on chitosan and β-cyclodextrin to improve warfarin solubility and stability and to achieve a more controlled drug release profile. The study began with the formation of a warfarin–cyclodextrin inclusion complex using the ionotropic gelation method, which was subsequently incorporated into a chitosan matrix to form a drug delivery system. The resulting nanoparticles had an average particle size of 63 ± 23 nm with a high encapsulation efficiency of 94%. Drug release followed the Higuchi model, while ex vivo studies demonstrated that approximately 60% of warfarin permeated the skin within 8 hours, following zero-order kinetics. Drug release occurred consistently without significant fluctuations. Physicochemical characterization using FTIR confirmed the formation of interactions between the C=O group of warfarin and the NH_2 groups of chitosan, while XRD analysis indicated molecular dispersion of warfarin within the chitosan matrix. These characterization studies were essential to confirm nanoparticle

formation, given the low solubility of warfarin as a major obstacle in developing controlled-release formulations. Overall, the chitosan-based system loaded with the warfarin–β-cyclodextrin complex successfully enhanced warfarin solubility and stability, provided controlled drug release, and showed potential as an alternative warfarin formulation with improved release and pharmaceutical characteristics compared to free warfarin [8].

Adsorption dan Sol-Gel

Parfenyuk and Dolinina (2017) developed warfarin–silica composites using adsorption and sol–gel methods to produce controlled-release systems for warfarin. In the adsorption approach, silica (UMS and PhMS) was first synthesized using D-fructose as a pore-forming agent, followed by warfarin adsorption. In contrast, the sol–gel method involved incorporating warfarin directly into a mixture of TEOS and organosilane precursors (PhTMOS or MTMOS). The adsorption method produced porous silica particles with two size populations (0.8 µm and 2.5 µm), and DSC analysis revealed that part of the drug remained crystalline, as evidenced by peak broadening at 159°C. In the sol–gel system using phenyl-modified silica, particles of approximately 2 µm were obtained, and DSC indicated that warfarin was present in an amorphous form without melting peaks. FTIR analysis confirmed the presence of warfarin characteristic peaks within the silica matrix. Drug release testing showed a pronounced burst release (>70% within 8 hours) for adsorption-based systems, whereas sol–gel-derived systems exhibited slower and more stable release for up to 48 hours, approaching zero-order kinetics. These results demonstrated that particle morphology, silica type, and preparation method strongly influence the release rate and mechanism of warfarin [22].

Oil-in-Water Differential Microemulsion Polymerization

Moustafa et al. (2013) developed MMA/HEMA copolymer nanospheres to evaluate the capability of differential microemulsion systems to produce stable nanospheres and to assess warfarin distribution and release from polymer matrices. Oil-in-water differential microemulsion polymerization was employed to synthesize MMA/HEMA copolymer nanoparticles using PVP and PEG, with in situ drug loading during polymerization. Sodium warfarin was dissolved in the aqueous phase, and polymerization was initiated using ammonium persulfate at approximately 65°C under a nitrogen atmosphere. Particle sizes ranged from 40 to 90 nm depending on the MMA/HEMA ratio, with encapsulation efficiencies exceeding 90%. FTIR analysis confirmed the presence of characteristic warfarin sodium absorption bands in drug-loaded nanospheres that were absent in blank nanospheres. Drug release was pH-dependent, with slower release at pH 1.2 (indicating stability under acidic conditions) and faster release at pH 7.4 due to increased polymer hydration and swelling. Importantly, no burst release was observed. These findings suggest that appropriate polymer formulation design can be used to regulate warfarin release rates according to target physiological conditions, potentially reducing fluctuations in systemic drug levels [23].

Chemical Reduction

Leopold et al. (2019) demonstrated that warfarin can function not only as an anticoagulant but also as a stabilizing agent and ligand for gold nanoparticles (AuNPs). Gold nanoparticles were synthesized using a 1% sodium warfarin solution as a reducing agent, yielding a purple-red colloidal solution with a pH of approximately 8, indicative of AuNP formation. Comprehensive characterization included particle size analysis, EDS, FTIR, and TEM, followed by cytotoxicity and cellular uptake studies to ensure that warfarin modification did not induce harmful toxicity. The results showed warfarin-

coated AuNPs with an average size of 54 ± 10 nm and predominantly polygonal (mainly hexagonal) shapes. TEM images revealed successful cellular internalization, with nanoparticles primarily localized in lysosomes and some freely distributed in the cytoplasm. EDS confirmed the presence of gold as well as carbon and oxygen from the warfarin coating, while FTIR spectra displayed characteristic warfarin bands. MTT assays indicated no significant reduction in cell viability at low concentrations (up to 37.5×10^{-13} M) for both cell lines tested, although HFL-1 cells were more sensitive than D407 cells. No significant difference was observed between 24- and 48-hour exposures in HFL-1 cells, suggesting that cytotoxic effects primarily occurred within the first 24 hours. These findings highlight warfarin's potential role not only as a drug but also as a colloidal stabilizer and mediator of cellular interactions with potential biomedical applications [24].

Self-Assembly

Msolli et al. (2017) developed warfarin-loaded nanoparticles based on poly((R,S)-3,3-dimethylmalic acid) (PDMMLA) copolymers to create a controlled drug delivery system. The nanoparticles were synthesized via polymer self-assembly in an aqueous medium, followed by warfarin loading during particle formation. Particle sizes ranged from 50 to 93 nm depending on the copolymer type. Encapsulation efficiency decreased with increasing drug loading, reaching approximately 95% at 3%, 90% at 6%, and 85% at 10% warfarin loading. Drug release was gradual over four weeks without burst release, with approximately 38% release for W-NP10 and 47% for W-NP40 formulations. Stability studies over 14–28 days showed an increase in particle size. DSC analysis indicated glass transition temperatures (T_g) ranging from −9°C to 50°C, suggesting that at body temperature (37°C) the polymer exists near or below T_g in an elastic state [25].

Building on the expanded functionality of warfarin reported by Leopold et al. (2019), Wang et al. (2026) explored warfarin as a redox modulator in ferroptosis-based cancer therapy rather than as an anticoagulant. Biodegradable sonodynamic polymer nanoparticles loaded with warfarin were developed through self-assembly between warfarin and a pseudo-conjugate polymer (BSD), followed by PEG stabilization. The study evaluated ROS generation, ferroptosis induction, and antitumor efficacy both in vitro and in vivo. NP-BSD and NP-BSD@WFR systems produced ROS only upon ultrasound (US) activation, with warfarin functioning to inhibit ROS elimination rather than directly increasing ROS production. NP-BSD@WFR combined with US exhibited the lowest IC_{50} values and synergistically enhanced cytotoxicity compared to NP-BSD + US or free warfarin alone. Ferroptosis induction was confirmed by significant downregulation of GPX4, specific suppression of VKORC1L1 in the combination group, a ~15% reduction in GSH, a 3.7-fold increase in MDA, and enhanced lipid peroxidation confirmed by C11-BODIPY fluorescence shifts. The highest apoptosis levels were observed in NP-BSD@WFR + US groups in both conventional assays and 3D tumor spheroid models. In vitro, ferroptotic cancer cells increased BMDC maturation ($CD80^+CD86^+$) to approximately 58%, indicating immunogenic cell death. In vivo studies showed dominant tumor accumulation at 36 hours, minimal off-target organ distribution, tumor inhibition rates up to 92%, significant necrosis and apoptosis without major organ damage, and enhanced systemic immune activation. These findings demonstrate that biodegradable nanoparticle systems enable controlled therapeutic activation while minimizing systemic anticoagulant risk [14].

Nanoprecipitation

Zagorodnikova XA et al. (2024) developed warfarin-loaded PLGA nanoparticles to modify warfarin biodistribution and limit tissue exposure, particularly because warfarin is teratogenic and capable of crossing the placenta. Nanoparticles were prepared by gradually adding the organic phase (PLGA and warfarin) into an aqueous phase containing PVA and ICG at 55°C with stirring at 300 rpm. The resulting particles ranged from 100 to 200 nm with an encapsulation efficiency of 63%. Release studies showed approximately 10% warfarin release within 90 minutes, followed by gradual release over approximately 70 hours. In vivo biodistribution studies demonstrated reduced fetal exposure compared with free warfarin while preserving therapeutic efficacy. Warfarin concentrations in organs remained below 1%, and fetal compartment levels were below 0.1% at 60 minutes post-administration, although tissue distribution increased with advancing gestational age. This study demonstrated the potential of nanoparticle technology to reduce systemic side effects without altering warfarin's chemical structure [6].

b.) Evaluations

Particle Size, Polydispersity Index (PDI), and Zeta Potential

The size of a particle represents a key parameter in nanoparticle characterization, as it plays a major role in determining nanoparticle absorption and biodistribution behavior [26]. The Polydispersity Index (PDI) is used to indicate particle homogeneity [10]. Zeta potential refers to the surface charge of nanoparticles and plays an important role in determining dispersion stability. Nanoparticle systems exhibiting high zeta potential values, whether positive or negative, are generally considered more stable. In contrast, low zeta potential values may lead to particle aggregation, flocculation, or clumping, which results in reduced stability of the dispersion [26]. Measurements of zeta potential and average particle size can be conducted using a Particle Size Analyzer (PSA), as reported by Parfenyuk and Dolinina (2017), or Dynamic Light Scattering (DLS), as used by Msolli et al. (2017), Zagorodnikova XA et al. (2024), Wang et al. (2026), and Moustafa et al. (2013). Zeta potential measurements alone may also be performed, or Laser Light Diffraction can be used, as demonstrated by Scala-Bertola et al. (2012).

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)

Fourier Transform Infrared (FTIR) spectroscopy is applied to identify functional groups present in a sample through the absorption of infrared radiation related to molecular vibrational energy. These functional groups can be used to analyze interactions occurring between components in a system, as reported in several previous studies such as Khalil et al. (2012), Moustafa et al. (2013), Parfenyuk and Dolinina (2017), and Leopold et al. (2019) [27]. ATR-FTIR is a practical analytical technique that allows non-destructive measurements with minimal sample preparation. Most samples can be placed directly onto the ATR internal reflection element (IRE) without the need for mixing with matrices such as Nujol or KBr, facilitating analysis across various sample types, including solids (powders, crystals, films) and solutions [8].

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

TEM and SEM are electron microscopy are commonly used techniques to observe to evaluate the morphological characteristics, shape, and size of the nanoparticles; however, they serve different primary functions. Transmission Electron Microscopy (TEM) involves electron transmission through very thin samples to visualize internal structures (2D micro/nanostructures) by generating high-resolution images at the atomic level, providing highly accurate structural information, as demonstrated in studies conducted by Parfenyuk and Dolinina (2017), Moustafa et al. (2013), Msolli et al.

(2017), Leopold et al. (2019), Wang et al. (2026), Zagorodnikova XA et al. (2024) [27]. In contrast, Scanning Electron Microscopy (SEM) utilizes reflected electrons to examine the surface features of thicker samples, producing three-dimensional (3D) topographical images, making it suitable for observing shape, texture, and surface structure, as reported by Scala-Bertola et al. (2012), and Khalil et al. (2012) [28].

Turbidity Measurement

Turbidity measurements are conducted to assess particle stability in solution and to examine how synthesis parameters affect the development of stable nanospheres, as described by Moustafa et al. (2013) [23], [29]. Turbidity is determined by monitoring changes in solution turbidity during the nanosphere synthesis process through light transmittance (T%) measurements using a UV–Vis spectrophotometer. As polymerization progresses and nanosphere particles form, light passing through the system becomes increasingly scattered, leading to higher turbidity values. These changes serve as indirect indicators of particle formation and variations in particle size or number within the system [8], [23], [29], [30]. Stable systems exhibit relatively constant turbidity values, whereas significant fluctuations indicate particle aggregation or instability [23].

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is an analytical technique used to obtain information related to the thermal characteristics of materials, including transition temperatures (T°t), such as melting temperature and glass transition temperature (Tg). Tg represents the point at which polymers transition from a rigid to a soft state and is used to assess polymer chain flexibility, while shifts in Tg indicate interactions among sample components [31]. DSC also provides information on enthalpy changes (ΔH), representing the heat absorbed or released during thermal processes. Endothermic peaks (positive ΔH) indicate heat absorption, commonly associated with crystalline or amorphous characterization, whereas exothermic peaks (negative ΔH) indicate heat release and are used to identify degradation temperature profiles [32], [33], [34]. DSC operates by comparing heat flow differences between a sample and a reference during heating, with thermogram peaks reflecting thermal transitions within the sample.

Scala-Bertola et al. (2012) reported that warfarin transformed into an amorphous state after encapsulation, evidenced by the absence of a sharp melting peak at 165.9 °C, while the Tg and Tm of PCL remained detectable, indicating polymer structural stability. Similarly, Parfenyuk and Dolinina (2017) showed that warfarin existed in an amorphous form using the sol–gel method, as indicated by the disappearance of crystalline melting peaks, whereas in the adsorption method, warfarin partially retained its crystalline form, shown by a broadened peak at 159 °C. Msolli et al. (2017) used DSC to characterize the thermal properties of PDMMLA polymers by determining Tg values to predict degradation and drug release behavior at physiological temperatures. The Tg values ranged from 5–31.7 °C, below body temperature, indicating that nanoparticles were in an elastic state, allowing water diffusion and polymer degradation, thereby supporting controlled and progressive warfarin release.

X-Ray Diffraction (XRD)

X-ray diffraction (XRD) is a widely applied non-destructive analytical method for distinguishing crystalline or amorphous phases of materials and to analyze structural changes after complex formation [35], [36], [37]. When a sample is exposed to X-rays, interactions occur between the incident radiation and the crystal lattice, producing diffracted beams that are detected, processed, and quantified. The diffracted intensities at various angles are plotted as diffraction patterns, which represent atomic arrangements and interplanar spacing unique to each crystalline phase [36]. Khalil et al. (2012) employed XRD to determine changes in warfarin crystallinity in warfarin–β-cyclodextrin inclusion complexes and chitosan particles loaded with these complexes.

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is an analytical approach employed to study molecular structure and dynamics by exploiting the magnetic properties of certain atomic nuclei, such as ^{1}H and ^{13}C, which possess spin and act as small magnets. These nuclei absorb radiofrequency energy at characteristic resonance frequencies when exposed to an external magnetic field, resulting in measurable energy transitions [38], [39]. The emitted energy as nuclei return to their ground state is recorded as an NMR spectrum, providing information on chemical environments, functional groups, atomic positions, and polymer or polyelectrolyte structures.

Msolli et al (2017) used NMR to confirm successful synthesis of PDMMLA copolymers based on benzylic and hexylic units via ring-opening polymerization. The presence of aromatic benzyl, ester, and hexyl chain signals confirmed copolymer formation, while signal integration enabled quantitative determination of monomer composition. The disappearance of benzyl signals after deprotection confirmed the formation of free carboxyl groups, which are essential for amphiphilic properties, surface charge, and nanoparticle performance as a warfarin delivery system.

Energy Dispersive X-ray Spectroscopy (EDS)

Energy-Dispersive X-ray Spectroscopy (EDS), as conducted by Leopold et al (2019), was part of elemental characterization to confirm the chemical composition of synthesized gold nanoparticles. The primary purpose of EDS was to verify the presence of elemental gold (Au^{0}) and to provide indirect evidence of warfarin acting as a capping agent on the nanoparticle surface. The presence of Au peaks confirmed gold nanoparticle core formation, while C and O signals supported FTIR results indicating warfarin binding to the nanoparticle surface [24]. Thus, EDS served as complementary evidence that the nanoparticles were not bare gold but warfarin-capped gold nanoparticles, consistent with their intended biological applications. The combined results of EDS, TEM, FTIR, and zeta potential measurements strengthened the conclusion that warfarin-capped AuNPs were successfully synthesized with stable and biologically relevant compositions [24].

Warfarin Encapsulation Efficiency

Entrapment efficiency studies conducted by Scala-Bertola et al (2012), Khalil et al (2012), Moustafa et al (2013), Msolli et al (2017), and Zagorodnikova XA et al (2024) aimed to assess the ability of nanoparticles to retain or carry warfarin within their matrices rather than merely mixing it physically. Higher drug entrapment indicates more effective warfarin incorporation within the nanoparticle system [8].

Release Study of Warfarin

In vitro release studies were conducted to evaluate how warfarin is released from CS/γ-PGA nanoparticles into the surrounding medium over time under different pH conditions relevant to normal tissues [8], [40], [41]. Release studies are essential for

understanding drug release rates and assessing therapeutic effectiveness and safety. Scala-Bertola et al. (2012) and Khalil et al. (2012) designed release tests according to administration routes to prevent excessively rapid drug release that could increase toxicity risk. Other studies by Parfenyuk and Dolinina (2017), Moustafa et al. (2013), Msolli et al. (2017), and Zagorodnikova XA et al. (2024) also used release studies to identify release mechanisms, such as burst release indicative of surface-bound drug, or to determine kinetic models such as Higuchi or zero-order release.

Determination of Warfarin Concentration

Zagorodnikova XA et al. (2024) utilized high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) to achieve precise and sensitive measurements of warfarin concentrations in various biological matrices. Biological samples, including plasma and organ tissues, were prepared via liquid–liquid extraction. The method was validated through the determination of the limits of detection (LOD), limits of quantification (LOQ), and linearity ranges [6]. Precision and accuracy parameters were evaluated using relative standard deviation and coefficient of variation values, confirming method reliability for pharmacokinetic and biodistribution studies. HPLC–MS enabled quantitative determination of warfarin levels in maternal and fetal tissues, allowing objective evaluation of the effects of PLGA encapsulation on warfarin distribution and exposure during pregnancy [6].

Analytical innovations

a.) Methods

Electrochemical Sensors via Co-precipitation

Beyond therapeutic applications, warfarin-related technological development has also focused on analytical approaches. Gholivand et al. (2015) developed Fe_3O_4 nanoparticles modified onto carbon paste electrodes (CPE) to investigate electrochemical detection mechanisms of warfarin and to evaluate the role of Fe_3O_4 nanoparticles in enhancing electrochemical response and detection sensitivity. Fe_3O_4 nanoparticles were synthesized through co-precipitation of $FeCl_2$ and $FeCl_3$ using NaOH at pH 10, and were incorporated into CPEs with an optimized composition of graphite powder, paraffin oil, and Fe_3O_4 (69.2:23.1:7.7% w/w). Warfarin quantification was carried out using square-wave anodic stripping voltammetry (SWASV) in 0.1 M phosphate buffer (pH 6.0), with an accumulation potential of 0.6 V for 50 seconds and a scanning range of 0.6–1.2 V. The study evaluated warfarin electrooxidation behavior, electrochemical kinetics, and determination in real samples, providing fundamental insights for sensor development. Fe_3O_4 modification enhanced oxidation current, reduced overpotential, and enabled accurate warfarin determination in pharmaceutical samples, confirming its role as an electron transfer mediator and electrochemical catalyst [42].

Nanocomposite-Based Electrochemical Sensors

Manjunath et al. (2025) further investigated nanomaterial-modified electrochemical sensors using Fe_3O_4 nanoparticles and ZnO@MWCNT nanocomposites to evaluate sensitivity, selectivity, and detection limits. ZnO@MWCNT nanocomposites were synthesized via precipitation and sonication, dispersed, and drop-cast onto glassy carbon electrodes (GCEs). Electrodes were characterized using physicochemical and electrochemical techniques to confirm structure and surface conductivity. Warfarin detection was performed using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in phosphate buffer at optimal pH. Sensor performance was evaluated in terms of sensitivity, detection limit, selectivity, reproducibility, and

stability, and was validated in serum and urine samples using the standard addition method. The results showed better detection performance than conventional electrodes, which was attributed to the increased active surface area and improved electron transfer kinetics. The sensor exhibited excellent selectivity, ultralow detection limits, wide linear ranges, and strong performance in biological matrices, indicating its potential for rapid and cost-effective warfarin monitoring [13].

Two-Step Co-Condensation Using a Soft-Template Approach

Farjadian et al. (2024) developed modified mesoporous silica as a warfarin adsorbent for toxicity management, functioning as a passive therapeutic agent through physicochemical mechanisms. Mesoporous silica was synthesized using surfactant-templated sol–gel methods, followed by TEOS hydrolysis and condensation around surfactant micelles, aging, washing, drying, and surfactant removal via calcination or extraction to produce highly porous silica with large surface area. Adsorption studies, in vitro warfarin reduction assays, and in vivo safety and efficacy evaluations demonstrated that mesoporous silica effectively adsorbed warfarin, reduced drug levels in biological systems, and improved toxicological outcomes without significant adverse effects. These findings suggest that nanomaterial-based adsorption strategies may represent an alternative approach for managing warfarin toxicity and overdose [43].

Collectively, these findings demonstrate that nano- and microtechnology-based approaches not only enhance the safety and therapeutic effectiveness of warfarin through formulation modification and advanced electrochemical sensing, but also expand its application spectrum into novel therapeutic and clinical domains.

b.) Evaluations

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

Farjadian et al. (2024) used TEM to observe and evaluate the morphological characteristics, shape, and size of nanoparticles [27]. In addition, Farjadian et al. (2024) and Gholivand et al. (2014) used SEM to analyze the shape, texture, and surface structure of samples using three-dimensional (3D) topographic images [28].

X-Ray Diffraction (XRD)

Manjunath et al. (2025) used XRD to confirm the formation of crystalline ZnO and to verify that combination with MWCNTs did not disrupt the crystal structure.

Raman Spectroscopy

Raman spectroscopy is used to identify functional groups based on molecular vibrations generated through inelastic scattering of laser light directed at a sample [44]. Raman spectroscopy enables rapid and sensitive identification using minimal sample quantities and is label-free, allowing analysis without modifying or damaging the sample [45]. Manjunath et al (2025) confirmed the presence of ZnO and MWCNTs and their interactions using Raman spectroscopy without causing detrimental chemical changes to their respective material structures.

Warfarin Adsorption

Adsorption studies by Farjadian et al. (2024) evaluated the ability of amine-functionalized mesoporous silica (MS–NH_2) to effectively bind warfarin as an antidote strategy. The studies assessed maximum adsorption capacity, gastrointestinal pH effects, and interaction mechanisms between warfarin and silica surfaces [43]. These tests are critical because the proposed therapeutic strategy focuses on rapid detoxification through drug binding in the gastrointestinal tract rather than controlled release. Results showed effective adsorption at both pH 7.4 and pH 1.2, although adsorption reached saturation more rapidly under acidic conditions due to amine group protonation [43].

Effectiveness Evaluation

Effectiveness tests were conducted in vivo to demonstrate that adsorption capability in the study by Farjadian et al. (2024) translated into reduced warfarin toxicity in the body. Evaluated parameters included International Normalized Ratio (INR), prothrombin time (PT), organ damage biomarkers (AST, ALT, bilirubin, and LDH), and histopathological examination of vital organs [43]. Results showed that oral administration of MS–NH_2 in warfarin-overdosed animals significantly reduced INR and PT values, with effectiveness comparable to vitamin K as the standard therapy [43]. Additionally, significant improvement in liver damage biomarkers was observed, with no evidence of tissue damage or particle accumulation in vital organs [43]. These findings confirm that MS–NH_2 is an effective and safe warfarin antidote through adsorption of excess drug in the gastrointestinal tract.

Clinical Translation & Safety

All studies reviewed shared the same clinical problem, namely the narrow therapeutic index of warfarin and the high risk of plasma concentration fluctuations, which can lead to increased adverse effects such as bleeding or therapeutic failure. Modifications of warfarin—such as PCL, chitosan–β-cyclodextrin, silica, PLGA, and PDMMLA, developed as drug delivery systems are intended to stabilize the pharmacokinetic profile, reduce C_max, prolong warfarin release, and enable biodistribution analysis to control systemic exposure of warfarin to tissues within the body, including the fetus. However, these approaches still face a major challenge from a safety perspective, as long-term release systems are difficult to rapidly terminate in the event of adverse effects, thereby posing a potential risk for drugs that require individualized dose adjustment, such as warfarin. On the other hand, based on existing studies, warfarin has been shown to provide benefits as a gold nanoparticle ligand or as a redox modulator in ferroptosis therapy, indicating the potential for further development to expand the functional role of warfarin beyond its use as a cardiovascular therapy. Nevertheless, safety issues such as nanoparticle toxicity, tissue accumulation, and off-target effects still require further investigation, and therefore these approaches currently remain at the preclinical experimental stage.

In addition to drug delivery contexts, approaches aimed at reducing the risks associated with warfarin use, such as the development of silica adsorbents as antidotes for warfarin poisoning and electrochemical sensor technologies to monitor warfarin levels, appear more readily applicable in clinical practice. This is because these approaches do not alter warfarin's mechanism of action as a drug, but instead help improve therapeutic safety by enabling faster monitoring or earlier management of adverse effects. Although promising, safety challenges remain, particularly those related to measurement accuracy and the ability of the system to specifically detect warfarin.

Therefore, although nanoparticle technologies offer promising solutions to overcome the pharmacokinetic limitations of warfarin, their implementation in clinical practice depends heavily on how well drug release can be controlled and how quickly therapy can be adjusted when adverse effects occur. This makes safety considerations and ease of clinical implementation the primary factors before such technologies can be further developed and applied in patients.

Limitations of the Review

Most of the reviewed studies, including those by Scala-Bertola et al. (2012), Khalil et al. (2012), Parfenyuk and Dolinina (2017), Moustafa et al. (2013), and Msolli et al. (2017), remain primarily focused on the preclinical stage, such as physicochemical

characterization, drug release profiling, and in vitro or ex vivo evaluations. Consequently, long-term clinical safety and efficacy assessments, including clinically relevant endpoints such as INR control and bleeding risk, have been limited. In addition, not all studies demonstrated optimal release profiles; for example, burst release was observed in the adsorption-based system reported by Parfenyuk and Dolinina (2017), while depot or long-acting systems generally lack the ability to rapidly terminate drug release. This represents a critical limitation, as warfarin has a narrow therapeutic index and requires rapid and individualized dose adjustment. Furthermore, although studies exploring alternative functions of warfarin, such as those by Leopold et al. (2019) and Wang et al. (2026), provide innovative concepts, their findings are largely limited to cellular and animal models, leaving the clinical relevance, benefits, and safety in patients uncertain. Similarly, although studies by Zagorodnikova XA et al. (2024) and Farjadian et al. (2024) suggest potential clinical applicability, the evidence is still confined to animal models, and further evaluation of long-term safety, interindividual variability in humans, and potential drug–drug interactions is required. Meanwhile, research focusing on sensing and monitoring technologies, such as those by Gholivand et al. (2015) and Manjunath et al. (2025), remains limited to laboratory-based or model systems, necessitating further validation of accuracy, reproducibility, and performance under real clinical conditions. Therefore, the primary limitations across the reviewed studies include the lack of comprehensive clinical data, insufficient long-term safety evaluation, and the ongoing challenge of achieving stable warfarin release while still allowing rapid dose adjustment or therapy discontinuation in response to adverse effects.

4. Conclusion

This review highlights that the main challenge in warfarin therapy remains its narrow therapeutic index, which necessitates strict monitoring and careful dose adjustment to avoid adverse effects. Among the therapeutic strategies reviewed, biodegradable polymer-based nanoparticle systems, particularly those employing nanoprecipitation and self-assembly approaches, emerge as the most promising platforms, as they consistently demonstrate improved pharmacokinetic control, enhanced stability, and reduced fluctuations in systemic exposure in preclinical models. In parallel, from an analytical perspective, electrochemical sensor platforms incorporating nanomaterials represent the most clinically ready approach, as several systems have already achieved limits of detection within the therapeutic monitoring range of warfarin and have been successfully tested in serum or blood with acceptable precision and recovery. To ensure clinical applicability, further development is required, including large-scale or commercial-level manufacturing, in vivo evaluation of key haemostasis parameters such as INR, prothrombin time, and bleeding risk, as well as validation of sensor systems using patient samples with comparison to gold-standard methods. These steps are essential to confirm that the developed technologies can be safely and effectively applied in warfarin therapy.

Acknowledgements:

The authors would like to express their gratitude to the supervisors for their guidance and valuable input during the preparation of this review article. The authors also acknowledge the support provided by the Faculty of Pharmacy in facilitating access to academic resources and literature databases.

Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this paper.

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