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Review

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APPLICATION OF ELECTROCHEMICAL ANALYTICAL METHODS IN THE DETERMINATION OF THE DRUG WARFARIN

New perspectives on analytical chemistry*

This article is dedicated to Prof. RNDr. Jiří Zima, CSc., on the occasion of his upcoming 70th birthday.

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Warfarin is an anticoagulant drug with a narrow therapeutic index. Its accurate determination is essential to ensure effective and safe treatment, as even small deviations can lead to serious adverse effects. A wide range of analytical methods have been developed for its determination, including spectrometric and separation techniques. Electrochemical methods are characterized by simple experimental setups and low costs, while enabling rapid analysis with high sensitivity and acceptable selectivity. Owing to these advantages, they represent an effective alternative for determining drugs and are used in clinical diagnostics, drug quality control, and environmental analyses of drug metabolites. This review discusses the physicochemical and biological properties of warfarin and compares various analytical approaches to its determination, with an emphasis on electrochemical methods.

Keywords: electrochemistry, drug analysis, warfarin, anticoagulants, voltammetry, amperometry

Contents

1. Introduction

Warfarin first appeared in the 1920s following a series of mysterious cattle deaths in North Dakota and Canada. It was found that the cause was the consumption of spoiled hay, in which molds converted naturally occurring coumarins into dicumarol, a compound with anticoagulant effects. The research was funded by the

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Wisconsin Alumni Research Foundation (WARF), after which the newly synthesized substance was named. Warfarin first entered the market in 1948 as a rodenticide. In 1954, in the form of a water-soluble sodium salt, it was also approved for human use as an anticoagulant drug under the commercial name Coumadin. Today, its analogs, known as superwarfarins, are used as rodenticides, while warfarin sodium is still used as an anticoagulant in clinical practice[1].

2. Warfarin

2.1. Physical and chemical properties

Warfarin, chemical name 4-hydroxy-3-(3-oxo-1-phenylbutyl)coumarin (CAS (Chemical Abstracts Service) registration number 81-81-2), is an indirect oral anticoagulant. It is an acidic substance with a dissociation constant of $pK_a \approx 5.6$. Warfarin is practically insoluble in water but highly soluble in alcohols and organic solvents. Its sodium salt is insoluble in organic solvents but highly soluble in water and ethanol, which is why warfarin sodium (WAR) is used as a drug. The structure of WAR is shown in Fig. 1; the molecular formula is $C_{19}H_{15}NaO_4$, and the CAS registration number of the sodium salt is 129-06-6. The molecular weight of the sodium salt is 330.3 g mol^{-1}. The structure consists of an aromatic coumarin nucleus with a hydroxyl group at position 4. At position 3, it is substituted with a benzylideneacetone chain containing a chiral carbon. WAR has two enantiomeric forms, *R*- and *S*-WAR, but a racemic mixture of these is used for pharmaceutical purposes[2].

WAR commonly occurs in two forms – crystalline, formed by a clathrate with propan-2-ol, and amorphous. Crystalline WAR is a white, odorless, hygroscopic powder. It has a stable and well-defined structure, which is why it is used in tablets. In contrast, amorphous WAR is less stable and sensitive to moisture but has higher water solubility and is therefore used as an active ingredient in suspensions[3].

2.2. Biological properties

The most common indication for WAR is the treatment and prevention of atrial fibrillation, other heart rhythm disorders, and associated thromboembolic complications. WAR is also used for the prevention and treatment of venous thrombosis and pulmonary embolism, for complications associated with heart valve replacement, and for the prevention of recurrent blood clots after myocardial infarction[4].

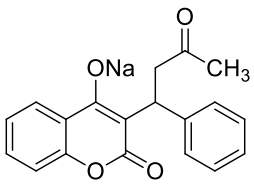

Fig. 1. **Structural formula of WAR**

The mechanism of action was first described in 1978, more than 20 years after its introduction to the market. The anticoagulant effect of WAR is based on the inhibition of vitamin K epoxide reductase (VKOR), an enzyme responsible for the regeneration of vitamin K in the liver. VKOR catalyzes the conversion of the oxidized form, vitamin K epoxide, to the reduced form, vitamin K hydroquinone, which is the active form. Active vitamin K is an important cofactor for the enzyme γ-glutamyl carboxylase, which ensures post-translational γ-carboxylation of coagulation factors II, VII, IX, and X, as well as the anticoagulant proteins C and S. Carboxylation allows these proteins to bind calcium ions, which is essential for their proper function. Uncarboxylated coagulation factors are biologically inactive and disrupt the coagulation cascade. WAR irreversibly binds to VKORC1, the catalytic subunit of the VKOR enzyme, leading to the gradual depletion of the active form of vitamin K. Since the effect is not direct, the onset of action is delayed, and the full anticoagulant effect occurs within five to seven days. Fig. 2 schematically illustrates the mechanism of action of WAR[4–6].

WAR is administered mainly orally, has high bioavailability, and is almost completely absorbed in the gastrointestinal tract. It reaches its maximum plasma concentration within 1.5 to 4 hours. In the blood, it is approximately 99% bound to plasma proteins, mainly albumin, resulting in a small volume of distribution of 0.08–0.12 L kg^{-1}. WAR is a racemic mixture of the *S*- and *R*-enantiomers, with *S*-WAR being three to five times more potent and therefore having the primary influence on the anticoagulant effect[2,4].

Metabolism occurs predominantly in hepatocytes *via* cytochrome P450 (CYP) enzymes, with each enantiomer being metabolized through a different pathway. The primary metabolic route is oxidation to hydroxy derivatives. The main enzyme involved is CYP2C9, which catalyzes the oxidation of *S*-warfarin to 6- and 7-hydroxy-*S*-warfarin. The metabolism of *R*-warfarin is more complex and occurs mainly through the involvement of CYP1A1, CYP1A2, and CYP3A4 enzymes, leading to the formation of *R*-WAR hydroxy derivatives. With the participation of additional CYP isoenzymes, other oxidation products of both *S*- and *R*-WAR are also formed. To a lesser extent, *R*-WAR derivatives may undergo conjugation with glucuronides or sulfates, but this pathway appears to be negligible in humans. A minor pathway, independent of CYP enzymes, is the reduction of the keto group to alcohol metabolites[2].

WAR is almost completely metabolized, and only a minimal amount is excreted unchanged. It is eliminated mainly in the urine (approximately 80%) and to a lesser extent in the feces (approximately 20%). The elimination half-life is highly variable and is influenced by many

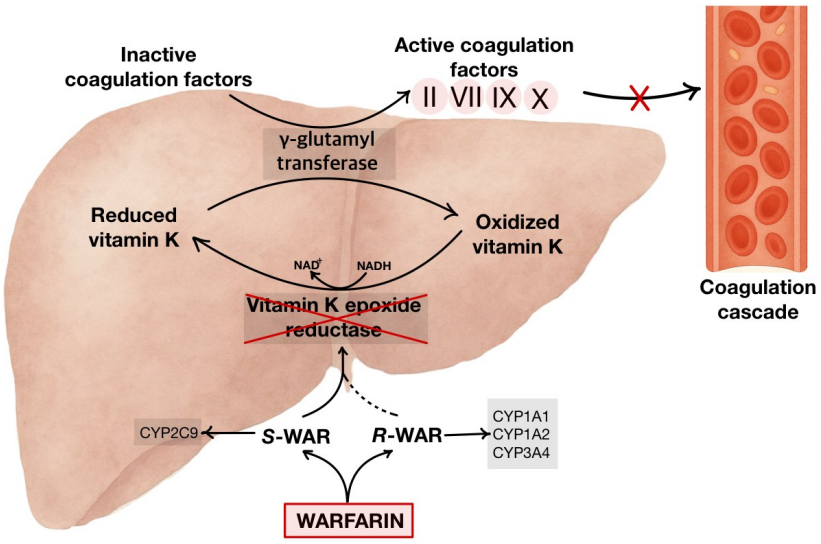

Fig. 2. **Mechanism of action of WAR in the body**

individual factors. For *S*-WAR, it ranges from 24 to 33 hours, whereas for *R*-WAR it ranges from 35 to 58 hours. Since *R*-WAR has a longer elimination half-life, it contributes to maintaining a more stable plasma concentration between doses. WAR crosses the placental barrier and is therefore not recommended during pregnancy – especially in the first and third trimesters – due to the risk of teratogenicity. In contrast, it does not pass into breast milk in clinically significant amounts and is therefore considered safe during breastfeeding[2,5].

As WAR is a drug with a narrow therapeutic index and high interindividual variability, dose adjustment is very challenging. Especially during the initial phase of treatment, complications may arise due to insufficient coagulation or, conversely, excessive blood thinning with an increased risk of bleeding. The international normalized ratio (INR) is used to monitor WAR dosing. It is calculated as the ratio of the prothrombin time – defined as the time required for clot formation in a blood sample after the addition of a reagent – to a reference time. A lower value indicates increased blood clotting. The physiological INR value is approximately 1.0, whereas during warfarin therapy it typically ranges from 2.0 to 3.0, or even higher[7].

From a genetic perspective, this variability is mainly influenced by polymorphism in two genes, namely for the aforementioned VKORC1 and CYP2C9 enzymes. These affect both the rate of metabolism and the body's sensitivity to WAR. Clinical factors include age, sex, body weight, comorbidities, and concomitant medications. Environmental factors and the patient's dietary habits are also important. One of the key factors is dietary vitamin K intake, which directly affects the efficacy of WAR. Other contributing factors include smoking, alcohol consumption, and the gut microbiome[5].

The need for constant INR monitoring and the risk of serious adverse effects have led to the development of a new generation of oral anticoagulants, known as direct oral anticoagulants (DOAC). DOACs exhibit fewer food and drug interactions, simpler dose adjustment, and a lower risk of internal bleeding. Despite these advantages, warfarin remains the only approved option for oral anticoagulant therapy in certain patient populations and indications, such as patients with mechanical heart valves or renal insufficiency. In addition, DOAC therapy is substantially more expensive, which represents a major limitation in clinical practice[8].

2.3. Electrochemical properties

The electrochemical oxidation of WAR is an irreversible process in which the hydroxyl group on the coumarin nucleus plays a key role. This group is also important in terms of the biological activity of WAR, as it interacts with the VKORC1 enzyme. To a certain extent, electrochemical methods can therefore be used to simulate processes occurring in the body.

A schematic diagram of the mechanism described in the literature[9] is shown in Fig. 3. Oxidation proceeds as a two-step process involving the transfer of two electrons. In the first step, the hydroxyl group is oxidized to a highly reactive radical. In the second step, this radical rapidly undergoes further oxidation to form a quinone structure that is stabilized by π-electron conjugation.

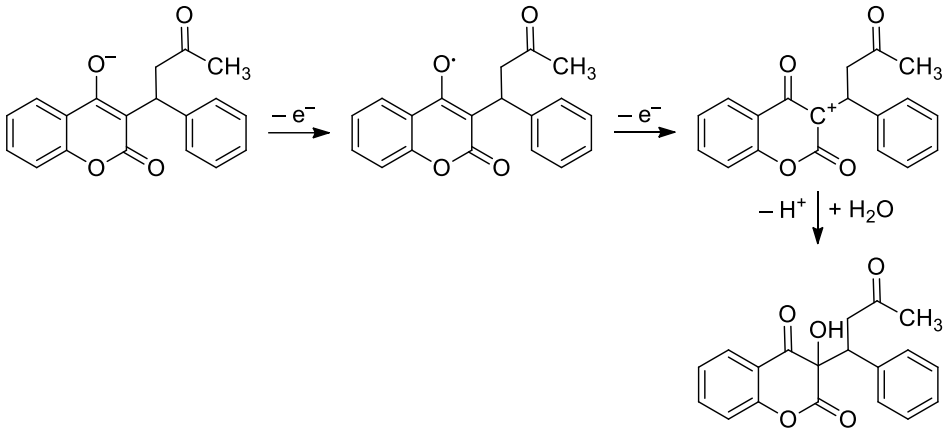

Fig. 3. **Scheme of the electrochemical oxidation of WAR**

3. Determination of warfarin

Given the risk of serious adverse effects and the narrow therapeutic index, reliable WAR determination is of great importance. In recent years, there has been growing interest in involving patients in monitoring their own health, which has encouraged the development of point-of-care (POC) devices that enable rapid and simple monitoring of WAR levels in blood. Accurate and reliable determination of WAR in pharmaceutical samples is also essential to ensure their quality and stability. Therefore, the development of rapid, affordable, and sensitive methods is of great importance.

3.1. Non-electrochemical methods of determination

The most commonly used methods include chromatographic and spectrophotometric techniques. In terms of sensitivity, selectivity, and the ability to separate enantiomers, high-performance liquid chromatography (HPLC) combined with UV-Vis spectrophotometry, fluorimetry, or mass spectrometry as a detection technique is dominant. An example is a method for the simultaneous determination of 18 substances, including WAR, using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The study analyzed whole blood, plasma, and less common samples such as vitreous humor and bile. A detection limit of 19.7 nmol L^{-1} was achieved (calculated based on a stated limit of quantification of 20 ng mL^{-1}). The advantages of this method include low sample consumption and simple sample preparation. Several substances of similar nature can be determined simultaneously in a very short time, making it suitable for forensic toxicology[10].

Chromatographic methods can be used to separate and quantify individual enantiomers. For example, one study employed HPLC with fluorescence detection. Detection limits of 0.067 ppm (0.22 µmol L^{-1}) for *R*-WAR and 0.090 ppm (0.29 µmol L^{-1}) for *S*-WAR were achieved. The method is reliable, suitable for routine monitoring, and has been applied to determine blood plasma levels in patients undergoing warfarin therapy[11].

Another application of these methods is the monitoring of drug interactions in pharmacokinetic studies. Using RP-HPLC with UV detection, a method was developed for the simultaneous determination of warfarin, aspirin, and clopidogrel, which are often used in combination in so-called triple antithrombotic therapy. The method exhibited good linearity over a concentration range of 5–200 µg mL^{-1} (16–650 µmol L^{-1}). A detection limit of 4.9 nmol L^{-1} was achieved (calculated based on a stated limit of quantification of 5 ng mL^{-1}). The method was also evaluated from the perspective of green analytical chemistry, where it produced satisfactory results[12].

Other chromatographic methods are also employed. Gas chromatography with electron ionization and tandem mass spectrometry (GC-EI-MS/MS) has been used to determine WAR and its five hydroxy derivatives generated *via* the Fenton reaction. Linearity was achieved over a concentration range of 26–1543 ng mL^{-1} (84–5005 nmol L^{-1}), with detection limits for individual analytes ranging from 18.7 to 67.0 ng mL^{-1} (60.7–217 nmol L^{-1}). As this method allows the determination of both WAR and its degradation products, it is suitable for environmental monitoring, for example in wastewater samples[13].

An interesting and novel alternative for WAR determination is presented in a study using a fluorescent sensor based on nitrogen-doped carbon quantum dots (NCDs). These NCDs were synthesized from biomass *via* a simple and rapid microwave-assisted method, and the approach is also compatible with green chemistry principles. To improve selectivity, the sensor was combined with a device employing a machine learning algorithm, allowing determination even in complex or previously unknown samples. The WAR detection limit reached 0.039 µmol L^{-1}. The sensor was successfully applied to determine WAR and its metabolites in blood plasma and stagnant water samples[14].

Spectrophotometric methods are particularly suitable for determining WAR in pharmaceutical samples, for instance for quality control during production. These methods are also included in pharmacopoeias, including the Czech Pharmacopoeia[15], as official methods for determining the content of the active substance. Due to the strong absorbance of WAR, spectrophotometric methods are sufficiently sensitive and reproducible and are therefore widely used. However, they are not suitable for complex biological matrices or in cases where it is necessary to distinguish individual WAR enantiomers.

3.2. Electrochemical methods of determination

Electrochemical methods represent a suitable alternative to chromatographic and spectrophotometric methods and are receiving increasing attention. Their main advantages include a simple experimental setup, straightforward operation, and low cost, making them particularly suitable for smaller laboratories. In most cases, no complex sample preparation is required. These methods are less demanding in terms of chemical consumption, which results in reduced waste generation and enhances sustainability in accordance with the principles of green chemistry. Another important analytical advantage is their rapid analysis, combined with high sensitivity and selectivity. A further major advantage is the potential for miniaturization, enabling their use in portable devices suitable for field measurements. Owing to these properties, electrochemical methods are employed across a wide range of analytical applications, particularly in pharmaceutical and environmental analysis, the food industry, and clinical diagnostics.

The choice of electrode material plays a critical role in the development of electroanalytical methods. Unmodified electrodes are often preferred due to their simplicity, affordability, and sufficient sensitivity for many applications. For example, Hasmemi *et al.*[16] developed a simple, inexpensive, and sensitive sensor based on a carbon paste electrode (CPE). Under optimal conditions, a linear concentration range of 30–3000 μmol L^{-1} and a detection limit of 0.315 μmol L^{-1} were achieved. The sensor exhibited high selectivity toward WAR even in the presence of potential interferents, such as sodium and chloride ions or glucose. When applied to determine WAR in tablets, a relative standard deviation of 2.50% was obtained, indicating good reliability and reproducibility.

The unmodified electrode was also employed in the work of de Jesus Guedes *et al.*[17], who developed a method for WAR determination using batch injection analysis (BIA) and flow injection analysis (FIA) combined with multipulse amperometry (MPA) on a boron-doped diamond electrode (BDDE) with cathodic pretreatment. In a phosphate buffer solution at pH 7.0 with 10% ethanol, linear concentration ranges of 2–400 μmol L^{-1} and 4–400 μmol L^{-1} were obtained for BIA-MPA and FIA-MPA, respectively. The corresponding detection limits were 0.36 μmol L^{-1} for BIA-MPA and 0.51 μmol L^{-1} for FIA-MPA. Key advantages of these methods include simple operation, rapid analysis, and high throughput – 94 analyses per hour for FIA-MPA and 130 analyses per hour for BIA-MPA.

In another study, de Jesus Guedes *et al.*[18] developed a simple electrochemical kit for the determination of ten drugs with narrow therapeutic indices, including WAR. The kit employed a BIA-MPA assembly and a screen-printed carbon electrode (SPCE) in a phosphate buffer solution at pH 7.0 with 10% ethanol. For WAR, linearity was achieved over a concentration range of 2–100 μmol L^{-1}, with a detection limit of 0.07 μmol L^{-1}. The method allows reliable determination in pharmaceutical samples and can be miniaturized into a portable device.

Modification of the electrode can provide a larger active surface area, higher electrocatalytic activity, and more efficient electron transfer. Consequently, modified electrodes offer improved analytical properties, such as increased sensitivity, lower detection and quantification limits, extended linear ranges, and enhanced repeatability. Complex sample matrices, such as urine or blood, can introduce interferences, which can be mitigated through appropriate electrode modification, thereby increasing selectivity toward the target analyte. For example, Taei *et al.*[19] developed a CPE sensor modified with multi-walled carbon nanotubes and $ZnCrFeO_4$ nanoparticles. In a phosphate buffer solution at pH 4.5 containing sodium chloride, a wider linear concentration range of 0.02–920 μmol L^{-1} was achieved. The modification resulted in higher conductivity, better sensitivity, and a low detection limit of 0.003 μmol L^{-1}. The sensor was successfully applied to samples of commercial tablets, blood serum, and urine.

Since WAR is a racemic mixture, some studies have focused on developing sensors capable of distinguishing enantiomers. A CPE modified with 3,4,9,10-perylene-tetracarboxylic acid, which specifically interacts with WAR enantiomers, yielded a linear concentration range of 5–160 μmol L^{-1} and detection limits of 3.09 μmol L^{-1} for *R*-WAR and 4.02 μmol L^{-1} for *S*-WAR. The method was successfully applied to blood plasma and urine with recoveries of 97.1–102.9% (ref.[20]).

An example of potentiometric WAR determination is provided by Saeedi *et al.*[21], who developed an electrochemical sensor based on an ion-selective electrode (ISE) for direct WAR determination in blood. The sensor, designed for POC applications, enabled rapid and sensitive determination without the need for sample pretreatment. The developed sensor contained a polyvinyl chloride membrane with tetradodecylammonium chloride as an anion exchanger. Measurements were performed in a phosphate buffer solution at pH 7.4 and in whole blood. The sensor exhibited high sensitivity, with detection limits of 0.125 μmol L^{-1} in buffer and 14.1 μmol L^{-1} in blood, along with rapid response, good repeatability, and high selectivity toward interfering ions such as chloride and salicylate.

Other studies[22,23] investigated the electrochemical behavior of WAR on an SPCE and an ultratrace graphite composite electrode (UTGE). Methods using DC

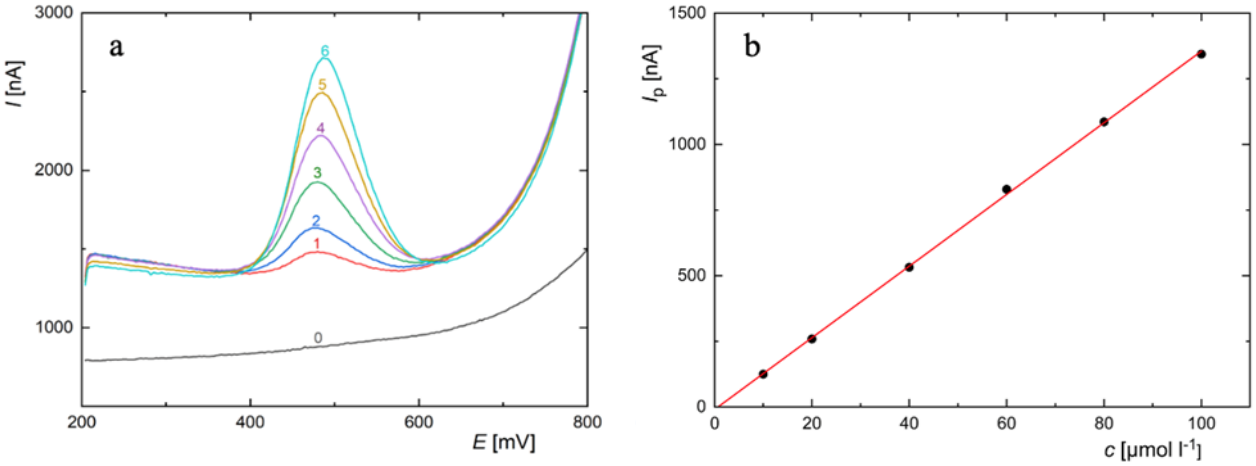

Fig. 4. **(a) DP voltammograms of WAR at concentrations of 0 (0), 10 (1), 20 (2), 40 (3), 60 (4), 80 (5), and 100 (6) μmol L^{-1} on an SPCE in a BR buffer at pH 7.0. (b) Calibration dependence of peak height (I_p) on WAR concentration.** DPV parameters: potential scan rate 20 mV s^{-1}, pulse height 50 mV, pulse width 100 ms (current sampled during the last 20 ms), pulse period 150 ms (ref.[22])

voltammetry (DCV) and differential pulse voltammetry (DPV) on an SPCE in a Britton-Robinson (BR) buffer solution at pH 7.0 provided linear ranges of 4–10 μmol L^{-1} for DCV and 10–100 μmol L^{-1} for DPV. Higher sensitivity was confirmed for DPV, with a detection limit of 0.26 μmol L^{-1}, whereas DCV exhibited a detection limit of 1.1 μmol L^{-1}. As an example, Fig. 4 shows voltammograms and the corresponding concentration dependence obtained on an SPCE using the DPV technique (ref.[22]). A UTGE combined with DPV in a BR buffer at pH 8.0 yielded a linear range of 8–200 μmol L^{-1} and a detection limit of 0.84 μmol L^{-1} (ref.[23]).

Further development involved an SPCE combined with batch injection analysis with amperometric detection (BIA-AD). Under optimal conditions, a linear concentration dependence in the range of 1–800 μmol L^{-1} was obtained, and a detection limit of 2.0 μmol L^{-1} was achieved. Fig. 5 shows the recorded amperograms and the corresponding concentration dependence. The key advantage of this method is its fast response and simple operation, which makes it particularly suitable for practical application. All the developed methods were successfully applied to determine the content of the active substance WAR in tablets, confirming their usefulness for pharmaceutical analysis[23].

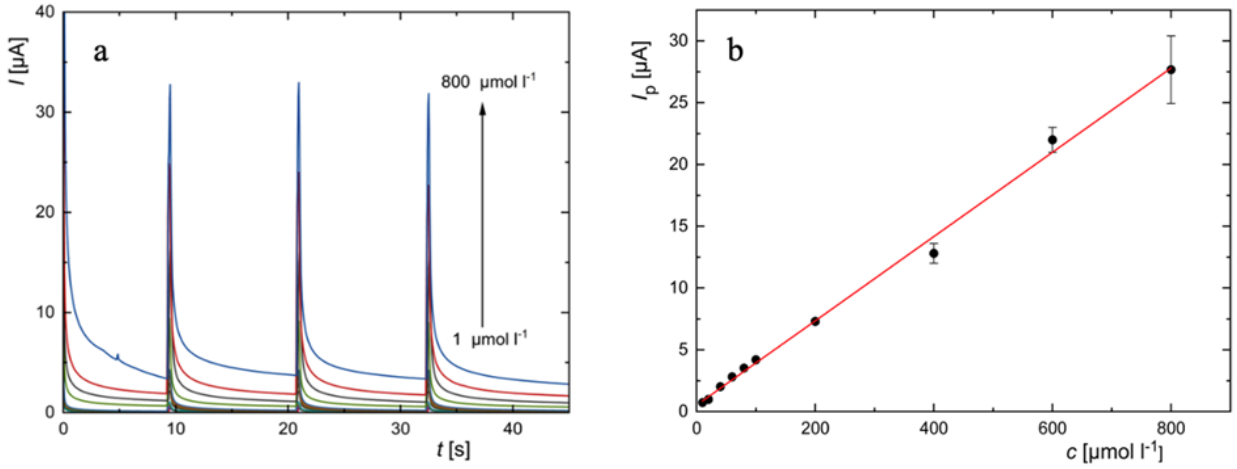

Fig. 5. **(a) Amperograms recorded using the BIA-AD technique on an SPCE in a BR buffer at pH 7.0 for a range of WAR concentrations of 1–800 μmol L^{-1}. (b) Calibration dependence of peak height (I_p) on WAR concentration;** error bars for n = 3. BIA-AD parameters: detection potential +1.0 V, dispensed volume 60 μL, dispensing rate 286.3 μL s^{-1} (ref.[23])

Table I
Overview of electrochemical methods for the determination of WAR

Working electrode	Technique	Medium	Linear range [μmol L^{-1}]	*LOD* [μmol L^{-1}]	Real samples	Ref.
CPE	DPV	PB (pH 4.5)	30–3000	0.315	tablets	16
HMDE	SWAdCSV	BRB (pH 5.0)	0.005–0.4	0.00065	tablets, serum, urine	24
Fe_3O_4/CPE	SWAdASV	PB (pH 6.0)	0.5–1000	0.21	tablets, serum, urine	9
$MnFe_2O_4$/MWCNT/CPE	DPV	PB (pH 4.0)	0.1–440.0	0.08	tablets, injection solution, serum, urine	25
$ZnCrFeO_4$/MWCNT/CPE	DPV	PB (pH 4.0)	0.02–920.0	0.003	tablets, serum, urine	19
β-CD/MWCNT/Co_3O_4NP/CPE	DPV	PB (pH 5.0)	0.05–150	0.02	urine, serum	26
CdS-QD/CS/MWCNT/GCE	DPSV	PB (pH 2.0)	0.05–80	0.0085	urine, serum, breast milk	27
Co_xO_y-NP/MWCNT/GCE	DPAdSV	PB (pH 6.0)	0.008–50 50–800	0.0033	urine, plasma	28
PTCA/CBPE	DPV	BRB (pH 7.0)	5–160	3.09 (*R*-WAR) 4.02 (*S*-WAR)	urine, plasma	20
Ni_3C/CPE	DPV	PB (pH 6.0)	0.008–1.5	0.0021	–	29
NiPILNF/GCE	SWV	PB (pH 7.0)	1–100	0.15	tablets	30
MIP/NPGL/GE	CV	PB (pH 7.5)	0.0001–0.08	0.00041	blood	31
$PrAlO_3$-NP/GCE	CV	PB (pH 7.2)	19.5–5000	19.5	water	32
ISE	potentiometry	PB (pH 7.4)	0.316–1000	0.125	blood	21
BDDE	BIA-MPA	PB (pH 7.0)	2–400	0.36	tablets	17
BDDE	FIA-MPA	PB (pH 7.0)	4–400	0.51	tablets	17
SPCE	BIA-MPA	PB (pH 7.0)	2–100	0.07	tablets	18
SPCE	DCV	BRB (pH 7.0)	4–100	1.05	tablets	22
SPCE	DPV	BRB (pH 7.0)	4–100	0.26	tablets	22
UTGE	DPV	BRB (pH 8.0)	8–200	0.84	tablets	23
SPCE	BIA-AD	BRB (pH 7.0)	1–800	2.0	tablets	23

Abbreviations not defined in the preceding text: *LOD* – limit of detection; PB – phosphate buffer solution; HMDE – hanging mercury drop electrode; SWAdCSV – square-wave adsorptive cathodic stripping voltammetry; BRB – Britton-Robinson buffer solution; Fe_3O_4/CPE – carbon paste electrode modified with Fe_3O_4 nanoparticles; SWAdASV – square-wave adsorptive anodic stripping voltammetry; $MnFe_2O_4$/MWCNT/CPE – carbon paste electrode modified with multi-walled carbon nanotubes and $MnFe_2O_4$; $ZnCrFeO_4$/MWCNT/CPE – carbon paste electrode modified with multi-walled carbon nanotubes and $ZnCrFeO_4$; β-CD/MWCNT/Co_3O_4-NP/CPE – carbon paste electrode modified with β-cyclodextrin, multi-walled carbon nanotubes, and Co_3O_4 nanoparticles; CdS-QD/CS/MWCNT/GCE – glassy carbon electrode modified with a chitosan film and multi-walled carbon nanotubes with covalently immobilized quantum dots; DPAdSV – differential pulse adsorptive stripping voltammetry; Co_xO_y-NP/MWCNT/GCE – glassy carbon electrode modified with cobalt oxide nanoparticles and multi-walled carbon nanotubes; PTCA/CBPE – carbon black paste electrode modified with 3,4,9,10-perylenetetracarboxylic acid; Ni_3C/CPE – carbon paste electrode modified with nickel carbide; NiPILNF/GCE – glassy carbon electrode modified with a poly(MImEO8BS)-Ni/Nafion nanocomposite; MIP/NPGL/GE – gold electrode with a molecularly imprinted polymer on a nanoporous gold film; CV – cyclic voltammetry; $PrAlO_3$-NP/GCE – glassy carbon electrode modified with perovskite nanoparticles.

An overview of electrochemical methods for determining WAR is given in Tab. I. The results show that electrochemical methods provide fast and sensitive options for determining WAR in a wide range of sample types. The combination with various materials and detection techniques allows for their further development and broader application in the field of analytical chemistry.

4. Conclusion

Even after the introduction of new generations of oral anticoagulants, warfarin remains of key importance in anticoagulant therapy. However, its narrow therapeutic index requires precise monitoring of blood concentrations, as insufficient dose control may lead to severe bleeding or, conversely, inadequate therapeutic efficacy. The determination of warfarin in biological and pharmaceutical samples is therefore crucial to ensure safe and effective treatment.

This paper provides an overview of developed electrochemical methods and their applications for the determination of warfarin. Electrochemical methods are characterized by high sensitivity, simple operation, low operating costs, and rapid analysis, making them an ideal tool for warfarin determination. Owing to modern electrode materials and the use of advanced detection techniques, these methods enable selective and accurate determination of warfarin even in complex biological matrices. Furthermore, the potential for miniaturization of electrochemical systems allows their use in point-of-care (POC) devices for rapid *in situ* warfarin analysis. These methods are therefore widely used in clinical practice for therapy monitoring as well as for quality control of pharmaceutical preparations.

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